SEARS FOUNDATION FOR MARINE RESEARCH
BINGHAM OCEANOGRAPHIC LABORATORY, YALE UNIVERSITY

JOURNAL OF MARINE RESEARCH

VOLUME IV, 1941

NUMBER 1

ISSUED APRIL 14, 1941, NEW HAVEN, CONN., U. S. A.

JOURNAL OF MARINE RESEARCH

PUBLISHED BY

SEARS FOUNDATION FOR MARINE RESEARCH
BINGHAM OCEANOGRAPHIC LABORATORY, YALE UNIVERSITY

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The Journal will be issued in three numbers per year, beginning with 1938. Volume I also contains the presubscription number (Vol. I, No. 1) issued in November, 1937.

Subscription price three dollars per year. Address all communications to the managing editor.

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MARINE RESEARCH

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POTENTIOMETRIC ANALYSIS OF SEA WATER
I. DETERMINATION OF CHLORINITY

By

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In general the determination of chlorinity of sea water is made by the classical Mohr method except when greater accuracy is desired in which case the more accurate but longer Volhard method is used. When the results are utilized for hydrodynamical considerations the Mohr method is normally used because of its speed in the titration of the numerous samples; but to attain the accuracy required in this exacting work, extreme care is demanded with the shaking during titration and in the proper selection of end point. A potentiometric determination seemed a convenient means of increasing the accuracy of the direct titration. For this purpose the apparatus recently described elsewhere by the authors (*2*) was used. It was found that the chlorinity could be determined potentiometrically with an accuracy and precision comparable to the Volhard (*1*) method in less time and with less labor than would be required by this latter method. As illustrative of the precision and accuracy of the potentiometric method, the following data are presented.

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SOLUTIONS

Silver Nitrate Solutions: Approximately 0.28 N $AgNO_3$ solution was preserved in a brown glass-stoppered bottle. Using a calibrated 25 ml. pycnometer its density was determined to be 1.03764 (in vacuo) at 20.0° C. A portion of the 0.28 N solution was diluted tenfold using calibrated volumetric apparatus.

Copenhagen Standard Sea Water: Two samples of standard sea water bottled at the Copenhagen Hydrographical Laboratories were used as standards. One was standardized in 1932 and had a chlorinity of 19.386‰ and the other in 1937 with a chlorinity of 19.393‰.

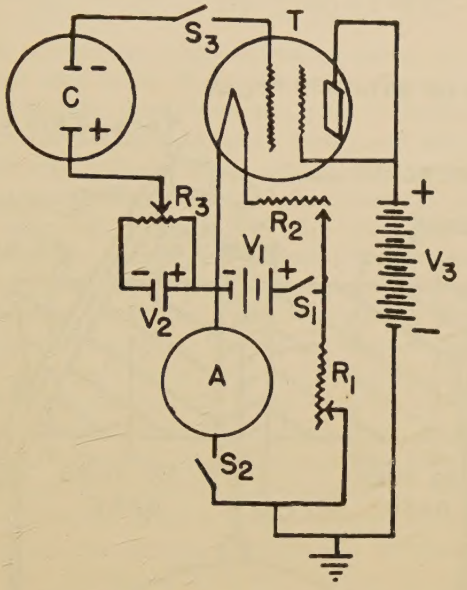
Standard Sodium Chloride Solution: A liter of solution contained 32.814 g. (in air) of purified and dried NaCl. Using a calibrated 25 ml. pycnometer its density (in vacuo) at 20° C. was determined to be 1.02110.

DESCRIPTION OF APPARATUS

The circuit diagram is shown in Figure 1. A Cunningham Radiotron vacuum tube, type 22, was selected because it seemed to have the most desirable characteristics (*3*) for the purpose at hand. The positive pole of the 2-volt battery V_2 was connected to the negative pole of the 4-volt battery V_1 thus allowing the 6-volt storage battery to serve a double purpose. As a precaution against drift and instability the vacuum tube and other parts easily affected by stray currents were inclosed in a copper screen-covered box, which was electrically grounded. Controls through a bakelite panel led into the box to operate the filament rheostat, a battery switch, the panel-type potentiometer and the control grid switch. The vacuum tube was placed inside the box in a stoppered glass cylinder in the bottom of which was placed a layer of desicchlora to maintain a dry atmosphere. The cylinder was closed with a rubber stopper through which passed the electrical leads from the tube socket terminals. The wire from the grid of the tube also passed through the stopper but was insulated from it by encasing in a ⅜″ glass tube and filling with sulfur. The control grid switch was mounted on the rubber stopper as shown in Figure 2. The top part of the glass tubing which carried the grid lead through the stopper was used as a cup for the mercury grid switch. A glass rod which projected through the bakelite panel could be rotated to operate the switch. A flexible insulated wire on the shaft of the switch led out of the box to the titration cell. The length of the grid lead from the tube to the titration cell was less than six inches.

Tests with the apparatus showed its free grid potential to be

— 0.23 volts and its voltage sensitivity to be 3.2 millivolts per microampere. Although this voltage sensitivity was adequate for the determination of the end point of the chlorinity titration, an increased sensitivity of 1.25 m.v. per unit scale deflection resulted



C	Titration Cell
T	Vacuum tube, type 22
A	Microammeter (scale 0-300)
V_1	4 volt storage "A" battery
V_2	2 volt storage "C" battery
V_3	45 volt dry "B" battery
R_1	5000 ohms
R_2	25 ohms
R_3	10,000 ohm panel-type potentiometer
S_1, S_2	Switches
S_3	Control grid switch

Figure 1. Vacuum Tube Voltmeter.

by substituting a galvanometer (Leeds and Northrup No. 2320 D, current sensitivity 5×10^{-7} amperes) for the microammeter (Weston D.C. Microammeter Model 1). The maximum current flowing through the grid while operating near the free grid potential was found to be less than 3×10^{-11} amperes.

A motor-driven glass stirrer was mounted above the small titration beaker. The electrodes were conveniently fastened to, and

insulated from, the frame of the motor stirrer. The electrodes were connected so that the increasing negative pole was attached to the grid lead of the vacuum tube. Natural graphite with a surface area of 4 to 6 sq. cm. served as the reference electrode and a 4 to 6 inch piece of No. 20 tungsten or platinum wire served as the indicating electrode.

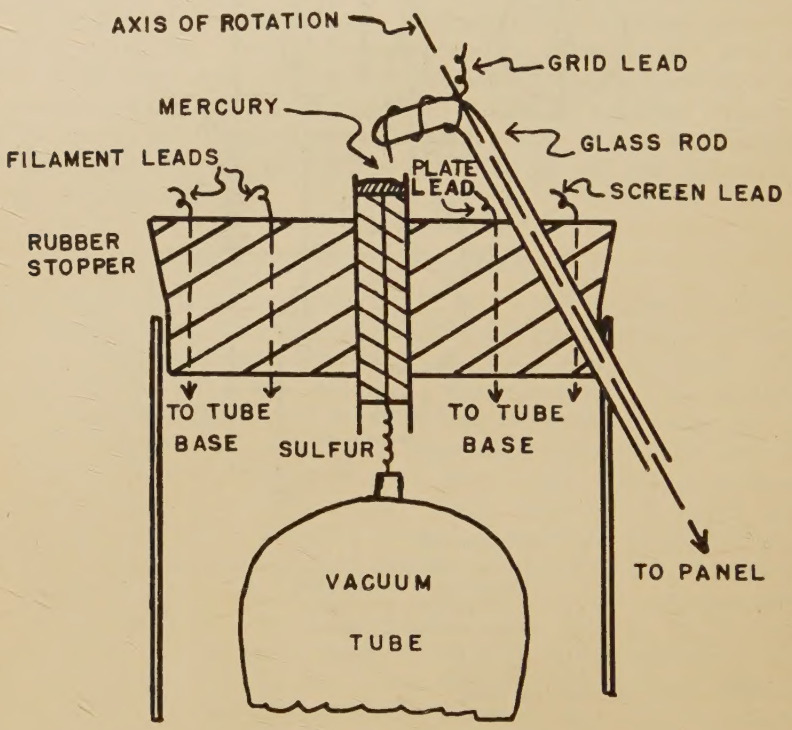


Figure 2. Control Grid Switch and Mounting.

OPERATION OF THE APPARATUS

S_1, see Figure 1, was closed and switches, S_2 and S_3, were left open. With the aid of a voltmeter, not shown in Figure 1, resistance R_2 was adjusted so that 3.3 volts was applied to the filament of the tube. Switch S_2 was then closed and by adjustment of R_1 the microammeter needle was set at a convenient value. The grid was then set at free grid potential by closing the switch S_3 and adjusting R_3 until the microammeter needle was again at its original setting. By

adjusting the grid to free grid potential before titration was begun and by connecting the electrodes in the manner indicated the grid was prevented from becoming positive during the course of the titration.

DISCUSSION OF APPARATUS

Vacuum tube voltmeters are especially useful in potentiometric titrations because they allow the course of a titration to be followed continuously. However, many vacuum tube voltmeters described in the literature are more elaborate, more costly and more sensitive than necessary for use with bimetallic electrodes in titrations of chlorinity. The apparatus as described here is characterized not only by its simplicity but also by its stability. Its free grid potential with respect to the negative end of the filament is less than 0.5 volt and at the beginning of a titration adjustment to this voltage is easily made in the manner described. The filament of the vacuum tube operates on only 0.13 ampere. Although the storage cells furnish a current of this magnitude with little fluctation the same storage cells are used to balance out the initial steady portion of the plate circuit. Thus any fluctation in the current from the filament battery is minimized in its effect on the microammeter in the plate circuit. Under the conditions of operation no fluctation of the microammeter needle could be detected over a thirty minute period. During the course of a titration the change of potential between the electrodes of the titration cell produced a corresponding change in the microammeter reading which was recorded rather than the more familiar e.m.f. reading. The microammeter reading may be easily converted to volts by calibrating the instrument but such a conversion is unnecessary since any function proportional to the voltage is equally well suited for the determination of the end point. In general only a few seconds were required for the potential between the electrodes to reach a steady state except near the end point when about a minute was allowed. Usually the end point was determined from the microammeter readings by inspection. However it may be obtained graphically by plotting the change in microamperes per unit volume of titrant against the corresponding volume of titrant and is very well defined when obtained in this manner.

STANDARDIZATION OF SILVER NITRATE

Procedure: About 25 ml. of the standard sea water or the sodium chloride solution were weighed into a 100 ml. beaker which was covered with a watch glass during the weighings to prevent excessive

evaporation. The weighings were recorded to the nearest milligram. A volume of 0.28 N silver nitrate was added so that a slight excess of chloride-ion still remained. The silver nitrate was weighed to the nearest milligram. The beaker was placed in the titration position, the electrodes inserted into the solution and the mixture stirred for several minutes before the titration was continued. The titration was completed by adding the dilute silver nitrate solution from a micro buret. With vigorous stirring of the solution the electrodes usually reached a steady state within a few seconds after each addition of silver nitrate solution. The entire titration usually did not require more than five minutes for completion.

Experimental Data: Potentiometrically definite and appreciable changes in the microammeter readings were noted with the addition of as little as 0.01 ml. of the 0.028 N silver nitrate solution when near the end point. The data for a typical titration are recorded in Table I. These data are shown graphically in Figure 3.

Since about 50 ml. of 0.28 N silver nitration solution were used in the titration and the error of establishing the end point was not

TABLE I

DETERMINATION OF THE END POINT IN THE SILVER NITRATE STANDARDIZATION

Mls. of Dilute $AgNO_3$	*Plate Current Microamperes*	*Microamperes per 0.05 ml. $AgNO_3$*
0.00	100	
1.00	105	
1.85	114	
		1.0
1.90	115	
		1.0
1.95	116	
		2.5
2.00	118.5	
		3.0
2.05	121.5	
		4.0
2.10	125.5	
		3.0
2.15	128.5	
		2.5
2.20	131	
		1.0
2.25	132	

End Point = 2.07 ml.

more than 0.01 ml. of 0.028 N solution, an error of not more than 1 part in 50,000 could have resulted. This is a sensitivity hardly attainable by a visual colorimetric end point. Such a great sensitivity suggests that chlorinity could be determined on micro samples by this method with considerable accuracy.

In the particular determination for which the data in Table I were obtained, 51.381 g. (equivalent to 49.514 ml.) of the silver nitrate solution had been added to 25.531 g. of Copenhagen standard sea water (Cl = 19.393‰) before the titration was completed with the

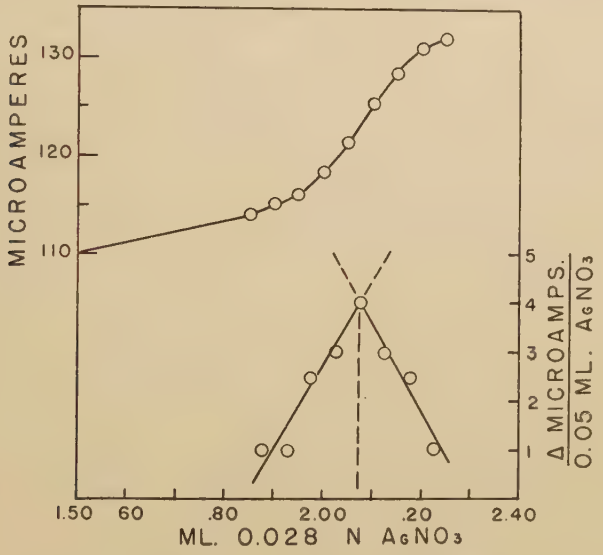


Figure 3. Titration of a Chloride Solution with Silver Nitrate.

dilute silver nitrate. The total volume of silver nitrate was then $49.514 + \frac{2.07}{10} = 49.721$ ml. Consequently the silver nitrate normality was $\frac{(25.531)\ (19.393)}{(49.721)\ (35.457)} = 0.28098$ with an error of not more than 1 part in 25,000 as far as the measurements were concerned. Results of other standardizations are shown in Table II. All weighings were corrected to in vacuo.

Discussion: These results listed in Table II are indicative of the precision that characterizes the potentiometric titration of chlorinity by the above method of determination. With one standard sea water

TABLE II

STANDARDIZATION OF SILVER NITRATE

Standard Sea Water	*Normality $AgNO_3$*	
	Uncorrected for atomic weights	*With new atomic weights**
Cl = 19.386 °/₀₀	0.28097	0.28112
	.28103	.28118
	.28096	.28111
	Ave. 0.28099	Ave. 0.28114
Cl = 19.393 °/₀₀	0.28094	0.28109
	.28096	.28111
	Ave. 0.28095	Ave. 0.28110
Standard NaCl Solution		0.28119
		.28114
		.28112
		.28119
		.28115
		Ave. 0.28116

*For recalculation with new atomic weights see (1).

the maximum deviation from the average was 1 part in 28,000, with the other sea water sample 4 parts in 28,000 or 1 part in 7,000. The average deviation was 1 part in 14,000. The difference between the average normalities as determined against the two sea water samples was 1 part in 7,000. Although this is a comparatively small variation it is somewhat greater than the experimental error of the determination. The accuracy of the potentiometric method as well as the Mohr and Volhard methods is limited largely by the amount of sorption of silver nitrate and sodium chloride by the silver chloride precipitate. With the Mohr method these occluded salts are brought into solution by manual shaking which at best is an indefinite and rather inefficient process. Moreover there is always some error in visual selection of the end point. With the Volhard method the excess silver nitrate is removed by washing of the silver chloride precipitate. Obviously only those salts on the surface are affected by the washing and those only partially since occluded salts can never be completely removed. Potentiometrically the salts need not be extracted but only brought to the surface to make them avail-

able for titration. Those salts within the silver chloride curds which are never exposed are not obtained by any method and so reduce the accuracy of the titration. It should be mentioned though that the mechanical stirrer is quite effective in the breaking of the silver chloride particles. Although the final accuracy of the determination may be more accurate than the standardization through a compensation of errors, it is desirable not to be dependent upon this stratagem whenever possible.

The potentiometric titrations may be made not only with great accuracy and precision but also with considerable rapidity. If the sample and silver nitrate solution are measured volumetrically as is done for the Mohr method the time for titration need not be much longer than would be required for the Mohr method while at the same time the accuracy would be greater. Since there is only one standard solution, no filtration and no washing of the precipitate in the potentiometric determination, the time is considerably less than for the Volhard method. If only the sample and the concentrated silver nitrate solution are weighed and the dilute solution measured volumetrically as is the procedure followed by the Copenhagen Laboratories in the preparation of the analyzed sea water samples, the weighings may be made very quickly and need not be particularly time consuming. If great accuracy is demanded the usual weight burets are essential but for most purposes the solutions may be weighed in a covered beaker. By making the weighings in the beaker, only three weighings are needed and by using a tared beaker and by measuring the sample and silver nitrate solution volumetrically into the beaker the approximate weights are already known and consequently little time is needed for final adjustment of the weights. A chainomatic balance is particularly appropriate for such routine weighings. Also by measuring and weighing the sample the approximate density and chlorinity of the water may be ascertained quickly so that the correct amount of the silver nitrate solutions may be added for weighing.

The titration of a sea water sample would be made in a manner identical to the above procedure for the standardization of the silver nitrate solution. Such titrations with this apparatus could be made aboard ship, particularly for titrations with only volumetric measurements of solutions. Obviously the use of a microammeter would make such titrations impractical on board ship in very rough seas. Consequently the greatest utility of the apparatus would be for very accurate work ashore.

The main disadvantage of course is that a special apparatus must be built and cannot be purchased as such. However the parts are either standard electrical equipment or may be purchased at radio

stores at a very small cost. The apparatus is relatively simple to construct and to operate.

SUMMARY

The results of potentiometric chlorinity titrations of sea water have been described using bimetallic electrodes and a vacuum tube voltmeter.

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DISSOLVED NITROGEN IN THE SEA WATER OF THE NORTHEAST PACIFIC WITH NOTES ON THE TOTAL CARBON DIOXIDE AND THE DISSOLVED OXYGEN

By

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This paper deals with a study and discussion of the distribution of dissolved nitrogen in sea water and its relation to the dissolved oxygen and the total carbon dioxide. Previous studies have been made by Buch (*1*), Knudsen (*8, 9*), Petterson (*11*), Jacobsen (*7*), Hamburg (*6*), Dittmar (*3*) and recently by Rakestraw and Emmel (*13, 14*). Fox (*4*) published data showing the variation in the concentration of dissolved nitrogen in equilibrium with the nitrogen of the atmosphere at standard pressure for different temperatures and for different concentrations of sea water. Using these data it is possible to calculate the percent of saturation of the dissolved nitrogen in sea water at a given temperature and chlorinity, the pressure of the gaseous nitrogen being equivalent to that of the gas in air at standard pressure. The inert gases, such as argon, were determined as nitrogen. Rakestraw, using a different method of analysis, obtained data that varies somewhat from the values of Fox, but he distinguishes between dissolved nitrogen and argon.

The determination of dissolved nitrogen is practically impossible to perform aboard ship because the motion of the ship makes it exceedingly difficult to secure accurate manometer readings.

Greenberg, Moberg and Allen (*5*) adopted the use of the Van Slyke apparatus for the determination of the total carbon dioxide in sea water and Moberg[1] first applied it for the determination of dissolved nitrogen. The accuracies of the determinations were increased by the use of an extraction chamber of 100 ml. and a sample of sea water of 10 ml. The Van Slyke apparatus has been used, with certain minor variations in technique, in securing the data reported in this paper. The details of manipulation of the apparatus have been described by Van Slyke (*16, 17, 18*) and coworkers and by Greenberg, Moberg and Allen (*5*). The observations consisted of measuring the changes in pressure at constant volume when the car-

[1] Private communication.

bon dioxide and oxygen were absorbed by reagents, the nitrogen being finally determined by expelling the residual gas. In the present study the term *dissolved nitrogen* thus refers not only to that gas but to all other inert gases and the data of Fox (*4*) are used in computing the percent saturation.

PROCEDURE

Determination of Total Carbon Dioxide

1 ml. of 1 N H_2SO_4 was introduced into the apparatus and the dissolved gases removed. 10 ml. of the sample of sea water were then placed in the apparatus using the special Van Slyke pipet. The acid decomposed the bicarbonates and the carbonates, liberating carbon dioxide.

The gases were extracted from the solution by lowering the mercury level and shaking for one minute; the gas volume was then brought to the 4 ml. mark and the reading of the manometer and the temperature of the water bath surrounding the extraction bulb, were noted. 0.4 ml. of 5 N KOH was placed in the extraction chamber to absorb the carbon dioxide, the mercury level lowered, the chamber shaken for one minute, the volume of the gas adjusted to 4 ml. and the manometer reading again noted. The amount of carbon dioxide present was then calculated using the following equation:

$$\text{mg. at. } CO_2/L = \frac{P(1000)ai}{s\ (760)\ (22.26)\ (1+0.00394t)}\left(1+\frac{S}{A-S}\alpha\right)$$

where

$$P = P_1 - P_2 - K_{CO_2}$$

P_1 and P_2 are the two manometer readings taken, and K_{CO_2} is an experimental constant determined by running a blank determination on 11 ml. of sea water which has previously been made alkaline with KOH. a, is the constant volume at time of measurement (4.0 ml.); s, is the volume of sample; 22.26 is the milligram molecular volume of CO_2; A, is the total volume of the extraction chamber; S, is the volume of sample plus the volume of reagent added; α is the distribution coefficient for carbon dioxide; t, is temperature of the water bath in degrees Centigrade; and i, is the experimentally determined factor for the reabsorption of carbon dioxide.

A value for the constant of Henry's law for carbon dioxide in water, 0.3372, has recently been determined by Shedlovsky and MacInnes (*10*). This value is undoubtedly of greater accuracy than 0.341 of Buch, et al (*2*). However the distribution coefficient of the latter investigators was used in calculating the results reported

herein. The difference obtained in substituting the value of Shedlovsky and MacInnes is so small as to be negligible.

Determination of Oxygen

After the total carbon dioxide had been determined, 1 ml. of oxygen absorbent was introduced into the extraction chamber.

The oxygen absorbent, as described by Van Slyke and Sendroy, (*17*) was prepared by grinding together 10 grams of sodium anthrahydroquinone-beta sulfonate and 100 grams of sodium hydrosulfite ($Na_2S_2O_4$). Five grams of this mixture were added to 25 mls. of 1 N KOH, the solution filtered and the gases extracted with the Van Slyke apparatus. The solution was then preserved under a Mohr seal of paraffin oil in order to prevent contact with the oxygen of the air.

After the oxygen had been absorbed the manometer reading was again taken and the amount of oxygen present calculated as follows:

$$\text{mg. at. } O_2/L = \frac{(2)P(1000)a}{s\ (760)\ (22.41)\ (1+0.00384t)}\left(1+\frac{S}{A-S}\alpha\right)$$

The symbols are the same as in the previous equation except that the milligram molecular volume of oxygen is 22.41; a is the distribution coefficient for oxygen, and $P = P_2 - P_3 - K_{O_2}$, where P_2 is the manometer reading after the absorption of CO_2, P_3 is the reading after absorption of oxygen and K_{O_2} is a constant which is determined by running a determination on 11.4 ml. of water from which the oxygen and carbon dioxide have been removed.

Determination of Nitrogen

The residual gases were expelled, the manometer again read and the milligram atoms of nitrogen calculated as follows:

$$\text{mg. at. } N_2/L = \frac{(2)P(1000)a}{s\ (760)\ (22.41)\ (1+0.00384t)}\left(1+\frac{S}{A-S}\alpha\right)$$

where the symbols are the same as in the oxygen equation except that a is the distribution coefficient for nitrogen, and

$$P = P_3 - Po - K_{N_2}$$

where P_3 is the manometer reading after absorption of oxygen, Po is the reading after expelling the residual gases, and K_{N_2} is a constant, which may be determined by running a determination using 12.4 ml. of water from which all dissolved gases have been removed.

The tables given by Fox for the solubility of nitrogen and oxygen

in sea water were recalculated in order to express the concentrations of the dissolved gases as milligram atoms per liter of water. These values are given in Table I for dissolved nitrogen and Table Ia for dissolved oxygen.

Determination of Chlorinity

The chlorinity was determined by the application of the Mohr method, the silver nitrate solution being standardized against the standard water of the Hydrographic Laboratories of Copenhagen, Denmark.

Experimental Errors

The maximum variation of the chlorinity determination never exceeded 0.012‰ Cl.

Using the Van Slyke apparatus, check determinations were always run for the total carbon dioxide and the dissolved oxygen and nitrogen. The pressure differences did not vary by more than 0.2 mm., or, in other words, the values always checked within 0.01 milligram atom for carbon dioxide and 0.02 milligram atom for nitrogen and oxygen.

EXPERIMENTAL

Surface Samples

During the summers of 1936 and 1937 samples of the surface sea water of San Juan Channel were collected daily at 8:00 A.M. from the observation pier of the Oceanographic Laboratories at Friday Harbor, State of Washington. The samples were immediately analyzed, using the methods outlined above, for total carbon dioxide and dissolved nitrogen and oxygen. The results of the analyses are given in Tables II and III.

In the tables the 1st column gives the date of sampling, the 2nd, the temperature of the water in °C., the 3rd column the chlorinity per kilogram of water; the 4th column the same values expressed per a liter of the water at 20° C.; the 5th, 6th and 7th columns, the concentration of total carbon dioxide, dissolved oxygen and dissolved nitrogen, respectively, the concentrations being expressed as milligram atoms of the element per liter of solution. The 8th and 9th columns give the percent saturation of the dissolved oxygen and the dissolved nitrogen. These values are obtained from Fox's data of the saturation of the gases in waters of varying chlorinities, different temperatures and at the partial pressure of the gases in the atmosphere at standard pressure. The 10th column shows the ratio between the total carbon dioxide and the dissolved nitrogen which is

obtained by dividing the value in the 5th column by that in the 7th. In the 11th column is given the carbon dioxide chlorinity ratio obtained by dividing the value in the 5th column by that in the 4th.

The waters of San Juan Channel tend toward homogeneity, as described by Phifer and Thompson (*12*), due to the marked turbulence resulting from the strong tidal currents and the erose nature of the channel bottom. At the entrance to the Strait of Juan de Fuca are waters that had their origin at depths varying from 100 to 400 meters in the Pacific Ocean. Due to a series of complex forces, these waters are forced to the surface and are carried into the Strait of Juan de Fuca where the strong tidal currents and the uneven bottom of the strait causes considerable mixing of the waters. The depths of the Strait of Juan de Fuca vary from 220 meters to about 20 meters, the average depth being about 150 meters. Part of this strait water flows into San Juan Channel which has an average depth of over 100 meters, where even stronger currents and uneven bottoms produce further mixing with the brackish water which has resulted from land drainage. The order of dilution of the original ocean water from drainage and rainfall varies from six to twelve per cent and of the original Strait of Juan de Fuca water from one to five per cent.

Examination of the data in Tables II and III shows a variation in the amount of dissolved nitrogen from 1.05 to 1.22 milligram atoms per liter with percent saturations ranging from 94% to 109%. The high saturations represented by the latter value, indicate a warming of the colder waters entering San Juan Channel from the Strait of Juan de Fuca. The concentrations of the dissolved oxygen varied from 0.31 to 0.57 milligram atoms per liter, the per cent saturation extending from 56% to 98% and averaging 80% for the summer of 1936 and 76.5% for 1937.

The waters are thus practically saturated with dissolved nitrogen but only partially with dissolved oxygen. As the waters of San Juan Channel very seldom approach saturation with oxygen, equilibrium with the air is never obtained. The high oxygen values are the result of photosynthetic reactions. Conditions of "supersaturation" of nitrogen occur with increase in the temperature of the water.

The concentration of total carbon dioxide was largely a function of the chlorinity of the water and varied from 1.76 to 2.04 milligram atoms of carbon per liter. The high values for total carbon dioxide were accompanied by low concentrations of dissolved oxygen, higher chlorinities and lower temperatures, characteristics of the sub-surface water of the Pacific.

The ratio between the total carbon dioxide and the dissolved nitro-

gen gave average ratios of 1.71 and 1.77 for the summers 1936 and 1937, respectively. Over half of the samples did not deviate from the average by more than 3%.

The maximum experimental errors in the methods of analyses would produce a variation in the total carbon dioxide-chlorinity factor of 0.0020. Approximately fifty per cent of the ratios are within this variation. The greatest deviations of the ratios occurred with waters of low chlorinity which gave higher ratios.

Oceanic Stations

Samples of water were taken beyond the continental shelf at various depths and several stations using 2.5 liter Nansen-Knudsen bottles. The samples for dissolved nitrogen were obtained in 250 ml. brown glass bottles from the sampling apparatus as soon as it arrived on board. The procedure for securing the samples was analogous to that for dissolved oxygen, the usual precautions to prevent contamination and contact with the air being followed. Each sample was then treated with 1 ml. of a saturated solution of mercuric chloride and then sealed under a mercury trap in much the same manner as later described by Rakestraw (*13*). The samples were stored under water at as low a temperature as possible to prevent the precipitation of gases and to keep them from becoming supersaturated with respect to the dissolved nitrogen. The samples were analyzed immediately upon reaching the shore laboratory. The results are given in Tables IV and V.

As most of the samples from the deeper water layers were low in dissolved oxygen, an excessive experimental error was introduced by the use of the Van Slyke method. Thus the dissolved oxygen values given in Tables IV and V were obtained by the Winkler method.

Two stations were selected for studying the distribution of the dissolved nitrogen. The first station was west of the Queen Charlotte Islands (Lat. 54° 10′ N., Long. 134° 10′ W.). The second station was beyond the continental shelf off the coast of the State of Washington (Lat. 48° 24′ N., Long. 127° 02′ W.).

At both stations the lowest values for dissolved nitrogen were obtained at a depth of 10 meters and low values were likewise secured in the samples taken from 20 and 30 meters at the station off the coast of Washington. From 10 meters to about 150 meters the samples all showed a per cent saturation less than that found at other depths.

Most of the dissolved oxygen occurring in the surface layers has originated from photosynthetic reactions. Minute bubbles of oxygen liberated by these reactions give rise to a two phase system in

which the pressure of oxygen is far greater than that of the partial pressure in the air. This causes the precipitation of some of the dissolved nitrogen as the waters are saturated in respect to this gas at the partial pressure of the nitrogen of the air. Thus in the photosynthetic zone the concentration of the dissolved nitrogen may be less than that in the water at or very near the surface or below the zone. The liberated particles of gaseous nitrogen dissolve in the water of the surface layer or else escape into the atmosphere.

The concentration of total carbon dioxide gradually increased with depth. The ratio between the total carbon dioxide and the chlorinity for the waters of both stations studied showed a maximum deviation of over ten per cent from the mean, a deviation far greater than would be occasioned by any experimental error. The lowest ratios occurred in the zone of photosynthetic activity and the highest were observed in the bottom waters. The lowest ratios at both stations existed at 10 meters.

The ratio between the carbon dioxide and the dissolved nitrogen did not vary by more than three per cent. The values are higher than those obtained for inshore waters but the ratio is much more constant. For the oceanic stations the average deviations are 0.033 and 0.026. Deviations of this order are only slightly greater than the possible experimental error, which indicates tendency toward the establishment of equilibrium in both the case of total carbon dioxide and nitrogen. The greatest deviations occur generally in the photosynthetic zone where the carbon dioxide is affected by biochemical action.

In Table VI the data of Rakestraw and Emmel for a station in the Atlantic is given for comparison. They do not give any data for 10, 20, and 30 meters and their concentrations of dissolved nitrogen are considerably lower for the Atlantic waters for depths between the surface and 1000 meters than the concentrations reported herein for the Pacific. However this would be expected because the temperatures between the depths just mentioned are much higher than those of the Pacific. The chlorinities of the Atlantic are also somewhat higher than those of the Pacific. Below 1000 meters the Atlantic concentrations for dissolved nitrogen are slightly less than those of the Pacific but the rather small differences may be due to different methods of analysis.

Nitrogen may be liberated by means of bacteria. Dr. B. S. Henry, formerly bacteriologist of the Oceanographic Laboratories, informed the authors that on 71 cultures isolated from the waters of the Puget Sound region and the deep waters beyond the continental shelf, he had found that 34 of the 71 strains reduced nitrates at least to nitrites. It was not determined whether any of these actually liberated

nitrogen. Zobell and Anderson (*19*) state that in most mud samples 5 to 15% of the bacteria are capable of reducing nitrates to nitrites, but few of them liberated nitrogen. Zobel and Feltham (*20*) also report two out of 20 nitrate reducers carried the reduction further but no statement was made as to whether this was molecular nitrogen or ammonia.

Nitrites will react with ammonium salts to give nitrogen as follows:

$$NO_2^- + NH_4^+ \rightarrow N_2 + H_2O$$

The nitrite ions occur only in the surface layers, the concentrations fluctuating with the season of the year. Nitrites are seldom found in depths greater than 200 meters although in deep fjords or inland passages, nitrites may occur. The concentration of ammonium ions, as shown by Robinson and Wirth (*15*), is exceedingly small, averaging less than 0.3 μg. at. for waters of the North Pacific below 100 meters and 1 μg. at. above this depth.

Nitrites also will react with the amino group in the presence of hydrogen ions:

$$-NH_2 + NO_2 + 2H^+ \rightarrow N_2 + 2H_2O$$

Robinson and Wirth have shown that albuminoid nitrogen exists in the North Pacific in concentrations approximating 3 μg. at.

From the above, it seems hardly probable that the dissolved nitrogen of the sea is materially affected by chemical or bacterial action. The dissolved nitrogen approximates 1.20 mg. at. while that from any above reactions would yield quantities of about 0.0003 to 0.001 mg. at. If the dissolved nitrogen accumulated in the sea, then evidence of "supersaturation" calculated from Fox's data would be manifested. This is not the case.

Two suggestions have been made for utilizing the information supplied by a knowledge of the concentration of dissolved nitrogen. One suggestion was that the amount of nitrogen found in the water at any depth gives an indication of the temperature of that water when it existed as surface water and became saturated with nitrogen due to exposure to air. Knudsen showed that this assumption was in error because of mixing of waters of various chlorinities and temperatures after exposure to the atmosphere. Buch suggested that the amount of nitrogen could be used to calculate the amount of oxygen that originally was dissolved with the nitrogen. By subtracting the amount of oxygen found at a particular depth from the calculated amount that probably entered with the nitrogen, the amount of oxygen that has been consumed is obtained. Negative values show that reactions which tend to produce an increase in the amount of oxygen

predominate over those that consume oxygen. Buch's calculations have been carried out for one deep sea station as shown in Table V. This calculation was based upon the assumption that the nitrogen was biologically and chemically inactive. The data presented above indicates that dissolved nitrogen is removed from the waters of the photosynthetic zone because the concentrations are smaller than those existing at the immediate surface and at other depths.

The fact that the per cent saturation of nitrogen is never far from 100 per cent (referred to saturation tables in which the pressure of the gas was the same as its pressure in the atmosphere) is an indication that the concentration of dissolved nitrogen is not considerably influenced by chemical or biological change. If the dissolved nitrogen found in the depths was largely the product of chemical and biological activity, then the concentrations existing should be greater because of increased pressures.

SUMMARY

1. The Van Slyke apparatus was used in modified form for the determination of total carbon dioxide, dissolved oxygen and nitrogen in sea water.

2. Surface waters at Friday Harbor, Washington, in the San Juan Archipelago were collected daily during the summers of 1936 and 1937 and analyzed for dissolved nitrogen.

3. Samples from several stations beyond the continental shelf were taken at various depths and analyzed.

4. Fox's table giving solubility of nitrogen and oxygen was recalculated to give solubilities in milligram atoms of nitrogen per liter of sea water.

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TABLE I

Solubility of Nitrogen in Milligram Atoms per Liter of Sea Water at Various Chlorinities, Temperatures, and at the Partial Pressure of Nitrogen in Air when Air Pressure is 760 mm. Mercury
[Calculated from Data C. J. J. Fox (*4*)]

Temp	*Cl°/₀₀ 13*	*Cl°/₀₀ 14*	*Cl°/₀₀ 15*	*Cl°/₀₀ 16*	*Cl°/₀₀ 17*	*Cl°/₀₀ 18*	*Cl°/₀₀ 19*	*Cl°/₀₀ 20*
0	1.435	1.416	1.398	1.378	1.359	1.340	1.321	1.302
1	1.406	1.388	1.368	1.350	1.332	1.313	1.295	1.276
2	1.376	1.360	1.340	1.323	1.304	1.287	1.269	1.251
3	1.349	1.331	1.314	1.296	1.280	1.261	1.244	1.226
4	1.321	1.305	1.288	1.271	1.254	1.237	1.220	1.204
5	1.295	1.279	1.263	1.246	1.230	1.214	1.199	1.182
6	1.270	1.255	1.238	1.223	1.207	1.192	1.176	1.161
7	1.247	1.231	1.216	1.201	1.186	1.170	1.155	1.140
8	1.223	1.208	1.194	1.179	1.165	1.149	1.136	1.121
9	1.200	1.187	1.172	1.158	1.145	1.130	1.116	1.102
10	1.180	1.166	1.153	1.139	1.126	1.111	1.098	1.084
11	1.159	1.147	1.133	1.119	1.106	1.094	1.081	1.067
12	1.140	1.126	1.114	1.101	1.088	1.076	1.069	1.050
13	1.121	1.109	1.096	1.084	1.071	1.058	1.046	1.033
14	1.104	1.091	1.079	1.064	1.056	1.043	1.031	1.020
15	1.085	1.074	1.063	1.051	1.039	1.027	1.016	1.004
16	1.070	1.058	1.047	1.035	1.024	1.014	1.002	0.991
17	1.053	1.042	1.032	1.021	1.010	0.999	0.988	0.977
18	1.037	1.027	1.017	1.006	0.995	0.985	0.975	0.965
19	1.023	1.013	1.003	0.993	0.982	0.972	0.962	0.952
20	1.015	0.998	0.989	0.979	0.969	0.959	0.949	0.939
21	0.995	0.985	0.976	0.968	0.958	0.948	0.937	0.927
22	0.982	0.971	0.963	0.954	0.945	0.935	0.925	0.915
23	0.969	0.960	0.951	0.942	0.934	0.924	0.915	0.905
24	0.957	0.949	0.940	0.932	0.923	0.913	0.904	0.895
25	0.945	0.936	0.927	0.920	0.911	0.901	0.893	0.884

TABLE Ia

SOLUBILITY OF OXYGEN AS MILLIGRAM ATOMS PER LITER OF SEA WATER AT VARIOUS CHLORINITIES, TEMPERATURES AND AT THE PARTIAL PRESSURE OF OXYGEN IN AIR WHEN AIR PRESSURE IS 760 MM. MERCURY
[CALCULATED FROM DATA C. J. J. FOX (*4*)]

Temp °C.	*Cl°/₀₀ 13*	*Cl°/₀₀ 14*	*Cl°/₀₀ 15*	*Cl°/₀₀ 16*	*Cl°/₀₀ 17*	*Cl°/₀₀ 18*	*Cl°/₀₀ 19*	*Cl°/₀₀ 20*
−2	0.825	0.815	0.804	0.792	0.781	0.768	0.758	0.748
−1	0.804	0.794	0.783	0.772	0.761	0.749	0.739	0.729
0	0.783	0.773	0.763	0.752	0.742	0.731	0.721	0.711
1	0.764	0.753	0.743	0.733	0.723	0.714	0.703	0.693
2	0.744	0.734	0.724	0.715	0.706	0.696	0.686	0.676
3	0.726	0.716	0.707	0.698	0.689	0.679	0.670	0.660
4	0.708	0.699	0.690	0.682	0.672	0.663	0.654	0.645
5	0.691	0.682	0.674	0.665	0.657	0.648	0.639	0.631
6	0.675	0.667	0.658	0.649	0.642	0.633	0.625	0.616
7	0.660	0.652	0.644	0.636	0.678	0.620	0.611	0.603
8	0.646	0.638	0.630	0.622	0.615	0.607	0.599	0.591
9	0.632	0.624	0.616	0.609	0.601	0.594	0.586	0.578
10	0.619	0.611	0.604	0.597	0.589	0.582	0.574	0.566
11	0.607	0.599	0.591	0.584	0.577	0.570	0.563	0.556
12	0.594	0.587	0.580	0.574	0.566	0.559	0.552	0.545
13	0.583	0.576	0.569	0.563	0.556	0.549	0.542	0.535
14	0.572	0.566	0.558	0.552	0.545	0.539	0.533	0.525
15	0.561	0.555	0.548	0.541	0.535	0.529	0.523	0.517
16	0.550	0.544	0.538	0.532	0.525	0.519	0.514	0.508
17	0.541	0.534	0.529	0.523	0.517	0.510	0.505	0.499
18	0.532	0.525	0.520	0.514	0.508	0.502	0.496	0.490
19	0.523	0.517	0.511	0.505	0.500	0.493	0.488	0.482
20	0.514	0.508	0.502	0.496	0.491	0.485	0.480	0.474
21	0.505	0.499	0.493	0.488	0.483	0.477	0.472	0.466
22	0.496	0.491	0.485	0.480	0.475	0.469	0.464	0.458
23	0.488	0.483	0.477	0.472	0.467	0.461	0.456	0.450
24	0.480	0.475	0.469	0.464	0.459	0.454	0.449	0.442
25	0.472	0.467	0.461	0.457	0.451	0.446	0.442	0.434
26	0.464	0.459	0.454	0.449	0.443	0.439	0.434	0.426
27	0.456	0.451	0.446	0.441	0.436	0.431	0.426	0.419
28	0.448	0.443	0.438	0.434	0.428	0.424	0.418	0.412
29	0.440	0.435	0.431	0.426	0.420	0.416	0.410	0.405
30	0.433	0.427	0.423	0.417	0.413	0.409	0.403	0.398

TABLE II

SURFACE SAMPLES, SUMMER 1936. SAN JUAN CHANNEL, SAN JUAN ARCHIPELAGO, STATE OF WASHINGTON

Date 1936		Temp. °C.	Chlorinity		Mg. At. per Liter			Per cent saturation		CO_2/N_2	CO_2/Cl
			°/oo	L_{20}	CO_2	O_2	N_2	O_2	N_2		
June	27	10.63	16.17	16.50	1.97	0.490	1.12	84.4	99.6	1.76	0.1194
	28	10.65	16.35	16.69	1.95	.533	1.21	92.0	106.5	1.61	.1168
	29	10.83	16.21	16.54	1.91	.568	1.14	98.0	102.0	1.71	.1154
	30	10.70	16.36	16.70	1.91	.528	1.17	91.8	104.4	1.69	.1144
July	1	10.65	16.39	16.73	1.92	.522	1.19	89.8	106.3	1.61	.1147
	2	10.80	16.42	16.76	1.93	.548	1.22	95.1	108.2	1.59	.1152
	3	10.35	16.57	16.92	1.95	.495	1.19	85.3	105.6	1.64	.1152
	4	10.46	16.67	17.02	1.96	.486	1.21	83.9	107.8	1.62	.1152
	5	10.10	16.72	17.07	1.98	.439	1.21	74.7	106.9	1.64	.1160
	6	10.00	16.84	17.20	1.99	.423	1.13	72.5	99.7	1.73	.1156
	7	9.95	16.85	17.21	2.02	.429	1.14	73.5	101.2	1.76	.1174
	8	10.32	16.84	17.20	1.96	.400	1.22	69.0	109.1	1.60	.1140
	15	10.28	16.72	17.07	1.99	.435	1.16	74.8	102.9	1.72	.1166
	16	11.82	14.73	15.00	1.78	.443	1.12	76.7	97.8	1.59	.1187
	17	11.22	15.82	16.13	1.95	.463	1.10	80.3	96.5	1.77	.1208
	18	10.70	16.41	16.75	1.98	.433	1.15	75.0	102.8	1.72	.1182
	19	10.65	16.38	16.72	1.95	.434	1.16	74.9	103.6	1.68	.1166
	20	10.59	16.58	16.93	2.00	.436	1.11	75.4	98.9	1.81	.1181
	21	10.45	16.58	16.93	1.93	.407	1.13	70.0	100.9	1.70	.1140
	22	10.50	16.72	17.07	1.91	.421	1.15	72.7	103.0	1.65	.1119
	23	10.65	16.72	17.07	1.96	.426	1.12	73.8	100.0	1.76	.1148
	24	10.47	16.75	17.10	1.99	.396	1.07	68.5	98.4	1.85	.1164
	25	10.68	16.71	17.06	1.90	.404	1.12	70.2	100.4	1.69	.1114
	26	10.79	16.53	16.88	1.92	.448	1.13	77.6	100.9	1.70	.1137
	27	11.14	16.09	16.42	1.90	.449	1.09	78.2	97.5	1.74	.1157
	28	12.13	15.40	15.70	1.87	.562	1.13	98.7	101.7	1.66	.1191
	29	12.20	15.41	15.71	1.80	.526	1.11	92.6	100.4	1.63	.1146
	30	11.83	15.80	16.11	1.81	.528	1.13	92.6	101.7	1.61	.1124
	31	11.50	16.06	16.39	1.85	.527	1.11	92.1	100.5	1.65	.1129
August	1	12.39	15.27	15.56	1.81	.508	1.15	89.7	104.4	1.57	.1163
	2	12.75	15.10	15.39	1.76	.502	1.09	89.1	98.8	1.62	.1144
	3	11.80	15.80	16.11	1.86	.469	1.07	82.3	96.8	1.72	.1156
	4	11.50	16.07	16.40	1.87	.459	1.10	80.3	98.4	1.69	.1140
	5	11.10	16.40	16.74	1.90	.456	1.09	79.5	98.1	1.75	.1135
	6	10.74	16.66	17.01	1.97	.457	1.10	79.4	98.2	1.79	.1158
	7	10.62	16.73	17.08	1.97	.431	1.10	74.6	98.1	1.80	.1153
	8	10.60	16.77	17.13	1.94	.411	1.09	71.4	97.7	1.78	.1133
	9	10.60	16.80	17.16	1.97	.430	1.11	69.9	98.9	1.78	.1148
	10	10.59	16.85	17.21	2.00	.398	1.12	70.0	100.4	1.78	.1162
	11	10.61	16.75	17.10	1.92	.432	1.10	75.0	98.4	1.75	.1123
	12	10.88	16.73	17.08	1.96	.435	1.12	75.8	101.0	1.75	.1148
	13	10.94	16.78	17.14	1.92	.450	1.14	78.6	102.8	1.68	.1120
	14	10.95	16.81	17.17	1.98	.448	1.12	78.2	100.6	1.77	.1153
	15	10.80	16.70	17.05	1.97	.460	1.11	79.9	99.2	1.78	.1155
	16	11.80	15.93	16.25							
	17	11.35	16.52	16.87	1.80	.490	1.04	86.0	93.7	1.74	.1067
	18	10.87	16.64	16.99	1.87	.434	1.07	78.6	95.8	1.75	.1101
	19	10.70	16.74	17.09	1.88	.438	1.08	76.0	96.4	1.75	1100
Average		10.93	16.37	16.71	1.918	.462	1.128	80.15	100.9	1.71	0.1149

TABLE III

SURFACE SAMPLES, SUMMER 1937. SAN JUAN CHANNEL, SAN JUAN ARCHIPELAGO, STATE OF WASHINGTON

Date 1937	Temp. °C.	Chlorinity		Mg. At. per Liter			Per cent saturation		CO_2/N_2	CO_2/Cl
		°/oo	$L_{20}°$	CO_2	O_2	N_2	O_2	N_2		
July 17	11.20	16.07	16.40	1.92	0.50	1.16	89	104	1.66	0.1171
18	11.12	16.11	16.44							
19	11.42	16.16	16.49							
20	11.03	16.30	16.64	1.94	0.55	1.15	98	105	1.69	.1166
21	10.68	15.77	16.08	1.95	.53	1.13	93	95.5	1.73	.1213
22	10.05	16.94	17.30	2.01	.45	1.13	79	95.4	1.78	.1162
23	10.41	16.73	17.08	1.98	.48	1.14	85	101.	1.74	.1159
24	11.35	15.89	16.21	1.91	.52	1.12	92	100.	1.70	.1178
25	11.38	15.85	16.16	1.89	.50	1.12	89	100.	1.69	.1170
26	11.13	16.22	16.55	1.95	.48	1.11	85	100.	1.76	.1178
27	11.02	15.91	16.23	1.93	.46	1.11	81	99.	1.74	.1189
28	10.90	16.36	16.70	1.95	.43	1.15	76	103.	1.70	.1168
29	10.58	16.64	16.99	2.01	.42	1.12	74	99.9	1.79	.1183
30	10.40	16.73	17.08	2.02	.40	1.13	71	101.	1.79	.1182
31	10.38	16.72	17.07	1.98	.41	1.12	72	100.	1.77	.1160
August 1	10.93	16.44	16.78	1.91	.43	1.10	76	98.6	1.74	.1138
2	11.05	16.53	16.88		.45	1.10	80	98.8		
3	11.07	16.53	16.88	1.89	.43	1.09	77	97.9	1.73	.1120
4	10.89	16.60	16.95	1.95	.42	1.05	75	94.5	1.86	.1150
5	10.70	16.74	17.09	1.97	.42	1.09	75	97.9	1.81	.1153
6	10.54	16.86	17.22	1.99	.42	1.12	74	100.	1.78	.1156
7	10.56	16.85	17.21	1.98	.42	1.10	74	98.5	1.80	.1150
8	10.47	16.90	17.26	1.98	.42	1.10	74	98.5	1.80	.1157
9	10.34	17.01	17.38	2.04	.41	1.12	73	99.8	1.82	.1174
10	10.37	16.99	17.36	2.03	.36	1.08	64	97.1	1.88	.1169
11	10.52	17.04	17.41	1.92	.37	1.09	66	97.2	1.76	.1103
12	10.64	16.99	17.36	1.93	.37	1.11	66	99.1	1.74	.1112
13	10.54	17.02	17.40	2.01	.37	1.06	66	94.6	1.90	.1156
14	10.69	17.00	17.39	2.00	.36	1.05	64	93.8	1.90	.1150
15	10.48	16.96	17.33	1.96	.42	1.09	75	97.2	1.80	.1131
16	10.80	16.72	17.07	2.01		1.10		98.8	1.83	.1178
17	12.01	15.14	15.43	1.91		1.10		98.7	1.74	.1238
18	12.72	14.69	14.96	1.88		1.10		99.8	1.71	.1257
19	12.20	15.63	15.94	1.92	.31	1.09	56	98.4	1.76	.1205
Average	10.90	16.47	16.81	1.94	.431	1.11	76.5	98.8	1.77	.1165

TABLE IV

DISTRIBUTION OF TOTAL CARBON DIOXIDE AND DISSOLVED OXYGEN AND NITROGEN IN THE WATERS OF THE PACIFIC, AUGUST 25, 1937. LAT. 48° 24′ N., LONG. 127° 02′ W.

Depth	*Temp.*	*Chlorinity*		*Mg. at. per Liter*			CO_2/N_2	*Per cent saturation*		CO_2/Cl
		L	°/₀₀	O_2	CO_2	N_2		O_2	N_2	
0	17.00	18.35	17.94	0.498	1.89	1.01	1.87	101.6	102	.1030
10	17.01	18.36	17.95	0.495	1.85	0.97	1.91	101.0	98	.1008
20	17.00	18.37	17.96	0.496	1.90	0.98	1.94	101.2	99	.1034
30	15.70	18.38	17.97	0.543	1.90	0.99	1.92	108.2	97	.1034
50	9.49	18.45	18.04	0.596	1.92	1.01	1.90	105.1	91	.1041
75	8.26	18.48	18.06	0.566	1.98	1.04	1.90	97.3	91	.1071
100	7.62	18.74	18.31	0.473	2.00	1.11	1.81	80.4	97	.1067
150	6.45	19.05	18.61	0.366	2.06	1.12	1.82	61.0	97	.1081
200	6.35	19.19	18.74	0.274	2.18	1.14	1.91	45.6	98	.1136
300	5.52	19.25	18.80	0.180	2.21	1.17	1.89	29.4	99	.1148
400	5.17	19.30	18.85	0.103	2.25	1.18	1.91	16.7	99	.1166
500	4.55	19.33	18.87	0.085	2.27	1.20	1.89	13.6	100	.1174
600	4.29	19.38	18.92	0.040	2.28	1.20	1.90	6.4	100	.1176
700	3.92	19.45	18.99	0.031	2.29	1.21	1.89	4.9	100	.1177
800	3.73	19.47	19.01	0.027	2.35	1.22	1.92	4.2	100	.1206
900	3.55	19.51	19.05	0.025	2.36	1.23	1.92	3.9	101	.1210
1000	3.34	19.51	19.05	0.022	2.38	1.22	1.95	3.4	100	.1220
1100	3.12	19.56	19.09	0.030	—	1.21	—	4.7	98	—
1200	3.01	19.56	19.09	—	2.39	1.22	1.96	—	99	.1222
1500	2.52	19.56	19.09	0.058	2.40	1.23	1.95	8.9	99	.1227
2000	1.90	19.66	19.19	0.119	2.41	1.25	1.92	17.9	99	.1226

TABLE V

DISTRIBUTION OF TOTAL CARBON DIOXIDE AND DISSOLVED OXYGEN AND NITROGEN IN THE WATERS OF THE PACIFIC, AUGUST 4, 1937. LAT. 54° 10′ N., LONG. 134° 10′ W.

Depth	Temp.	Chlorinity		Mg. at. per Liter			Per cent saturation		Mg. at. O_2/L with N_2	Mg. at. O_2/L used	% orig. O_2 rem.	CO_2/N_2	CO_2/Cl
		L	‰	O_2	CO_2	N_2	O_2	N_2					
0	13.59	18.37	17.96	0.511	1.99	1.01	97.8	96.1	0.498	−0.01	103	1.97	0.1083
10	13.48	18.39	17.98	0.553	1.93	0.99	105.	94.1	0.484	−0.07	114	1.93	0.1049
20	10.77	18.41	18.00	0.598	1.98	1.02	108.	92.8	0.504	−0.10	119	1.94	0.1076
30	8.67	18.44	18.03	0.583	2.00	1.07	101.	94.2	0.534	−0.05	108	1.87	0.1085
50	7.53	18.46	18.05	0.568	2.07	1.12	96.2	96.7	0.567	0.00	100	1.85	0.1121
75	6.97	18.59	18.17	0.504	2.09	1.10	84.4	94.2	0.550	0.05	92	1.90	0.1124
100	6.71	18.77	18.34	0.435	2.13	1.10	72.5	93.9	0.552	0.12	79	1.94	0.1141
150	5.84	19.10	18.66	0.336	2.17	—	55.2	—	0.543	0.21	62	—	0.1136
200	5.54	19.17	18.73	0.276	2.19	1.16	45.1	97.3	0.599	0.32	46	1.89	0.1142
300	5.03	19.27	18.82	0.179	2.26	1.18	29.0	98.3	0.604	0.42	30	1.92	0.1173
400	4.60	19.33	18.87	0.105	2.32	1.19	16.8	98.7	0.618	0.51	17	1.95	0.1200
500	4.36	19.35	18.89	0.076	2.35	1.19	12.1	98.0	0.618	0.54	12.3	1.97	0.1214
600	4.08	19.40	18.94	0.053	2.40	1.21	8.5	99.3	0.625	0.57	8.6	1.98	0.1237
700	3.96	19.42	18.96	0.047	2.38	1.21	7.4	99.0	0.625	0.58	7.5	1.97	0.1226
800	3.84	19.44	18.98	0.033	2.37	1.22	5.2	99.7	0.632	0.60	5.2	1.94	0.1219
900	3.65	19.47	19.01	0.030	2.38	1.20	4.7	99.0	0.619	0.59	4.8	1.98	0.1222
1000	3.50	19.50	19.04	0.026	2.44	1.22	4.1	100.	0.634	0.61	4.2	2.00	0.1251
1100	3.28	19 51	19.05	0.026	2.42	1.24	4.0	102.	0.648	0.62	4.0	1.95	0.1240

TABLE VI

DATA OF RAKESTRAW AND EMMEL SHOWING THE DISTRIBUTION OF DISSOLVED OXYGEN, NITROGEN AND ARGON AS MILLIGRAM ATOMS, IN THE WATERS OF THE ATLANTIC, JULY 12, 1937. LAT. 35° 30′ N., LONG. 67° 17′ W.

Depth	*Temp.*	*Cl*°/₀₀	O_2	O_2'	°/₀ *sat.* O_2	N_2	°/₀ *sat.* N_2	*Argon*
0	26.5	20.07	0.415	0.422	98	0.785	102	0.011
50	24.0	20.25	0.464	0.440	106	0.83	104	0.012
100	19.0	20.2	0.444	0.481	92	0.87	100	0.012
150	18.5	20.2	0.434	0.485	90	0.88	101	0.012
200	18.2	20.2	0.428	0.487	88	0.90	102	0.013
300	17.8	20.2	0.419	0.491	86	0.91	102	0.014
400	17.5	20.15	0.415	0.494	84	0.88	101	0.013
500	16.7	20.1	0.393	0.496	79	0.91	101	0.013
600	15.5	19.95	0.366	0.512	72	0.93	101	0.013
800	11.5	19.57	0.312	0.555	56	1.01	101	0.014
1000	7.5	19.42	0.366	0.602	61	1.10	101	0.015
1500	4.2	19.37	0.521	0.648	81	1.12	98	0.015
2000	3.5	19.35	0.553	0.658	84	1.13	97	0.015
2500	3.3	19.35	0.553	0.661	84	1.14	97	0.016
3000	3.0	19.32	0.544	0.667	82	1.14	96	0.016
3500	2.8	19.32	0.542	0.670	81	1.15	96	0.016
4000	2.5	19.32	0.535	0.675	79	1.155	96	0.016
4500	2.3	19.30	0.527	0.678	78	1.16	96	0.016

TABLE FOR DENSITY OF SEA WATER AT 0° C. AND ATMOSPHERIC PRESSURE FOR VALUES OF CHLORINITY BETWEEN 18.00 AND 20.99 ACCORDING TO KNUDSEN'S FORMULA

By

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It is customary to express the density of sea water at atmospheric pressure,

$$1 + \sigma_t \cdot 10^{-3},$$

in terms of σ_t to two decimal places. This is usually computed from chlorinity and temperature by means of the Hydrographical Tables (Knudsen, 1901), which give it as the difference of two terms:

$$\sigma_t = \sigma_0 - D.$$

σ_0 is given as a function of chlorinity alone, the density of sea water at 0° C. and atmospheric pressure being

$$1 + \sigma_0 \cdot 10^{-3},$$

and D is tabulated as a function of σ_0 and temperature. While D is tabulated to three decimal places, σ_0 is given to only two decimal places in the Tables, so their use is a departure from the common rule that calculations be executed to at least one more significant figure than the result.

Of course there is necessarily an uncertainty in the second decimal place of σ_t due to the uncertainty in chlorinity and temperature as commonly measured. This probably produces an error in σ_t which very often amounts to 0.01 and occasionally to even 0.03. Nevertheless this is no reason to introduce a further uncertainty due merely to calculation. This is especially true in deep water, where the second decimal place may assume great importance so that its highest accuracy in keeping with convenience is desirable. If σ_0 and D are known exactly to the second decimal place only, the resulting value of σ_t may be in error by as much as 0.010 and the chances are one in four that it is not correct in the second decimal place. Furthermore chlorinity is sometimes determined to some significance in the third

[1] Contribution No. 275.

decimal place (of ‰), and this cannot be adequately converted to σ_0 when the latter is tabulated to two decimal places only.

In order to eliminate largely this error of calculation we have formed the accompanying table giving σ_0 to three decimal places for the range of chlorinity from 18.00 to 20.99‰, which fully covers the waters found in the open ocean. It was computed from Knudsen's formula[2] relating σ_0 to chlorinity in parts per thousand,

$$\sigma_0 = -0.069 + 1.4708\,Cl - 0.001570\,Cl^2 + 0.0000398\,Cl^3.$$

In regard to the accuracy of the formula it is first to be noted that, even if the coefficients are exact in the last figure given, a maximum error in σ_0 of 0.0021 at chlorinity 20‰ might result because the subsequent figures are unknown. The coefficients were derived by Knudsen (1902, p. 157) from 22 selected samples for which the chlorinity and density were carefully determined. The average magnitude of the deviations of the σ_0 values from the formula is 0.0047; this was assigned chiefly to errors in the chlorinity determinations. The chlorinity values used by Knudsen, according to the old definition of chlorinity (Sørensen, 1902, p. 137) but using 1938 atomic weights, need to be increased by a factor 1.00048, the standard water of supposed chlorinity 19.380‰ having an actual value 0.0094‰ greater as given by Jacobsen and Knudsen (1940, p. 22). In order to obviate this discrepancy they have redefined chlorinity as 0.3285234 times the mass of silver necessary to precipitate the halogens in unit mass of sea water, and the formula for σ_0 is appropriate when chlorinity is thus defined.

A check on the formula at one point was obtained by the precise analysis of the Urnormal 1937 water. This sample of natural sea water was found to have a chlorinity of 19.381‰ and $\sigma_0 = 28.137$, the latter agreeing exactly with the formula (Jacobsen and Knudsen, 1940, p. 38). The conclusion indicated is that within the range of the accompanying table the third decimal place of σ_0 as given by the formula is doubtful but at the same time definitely significant even in absolute value. Of course it is assumed implicitly in the formula that variations in concentration of other dissolved substances relative to chlorine are too small to make the third decimal place of σ_0 insignificant. While this is justified for the samples Knudsen worked with, no sufficiently extended study has been made to ensure that the simple formula holds for all ocean waters to this degree of accuracy.

[2] Each value before rounding off to three decimal places was found to agree with the Hydrographical Tables with the exception of chlorinity 18.94‰, for which he gives $\sigma_0 = 27.49$, the formula giving 27.4952.

In regard to the values of D in the Hydrographical Tables, Knudsen (1902, p. 172) points out that linear interpolation may lead to an error as great as 0.002, but that the tabulated values are fully exact in the third decimal place. It is however doubtful that sufficiently diversified samples of sea water were investigated to be sure of this statement.

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TABLE OF σ_0 AS FUNCTION OF CHLORINITY

Cl,‰	.00	.01	.02	.03	.04	.05	.06	.07	.08	.09
18.0	26.129	143	158	172	187	201	216	231	245	260
18.1	26.274	289	303	318	332	347	361	376	390	405
18.2	26.419	434	449	463	478	492	507	521	536	550
18.3	26.565	579	594	608	623	637	652	667	681	696
18.4	26.710	725	739	754	768	783	797	812	826	841
18.5	26.855	870	885	899	914	928	943	957	972	986
18.6	27.001	015	030	044	059	074	088	103	117	132
18.7	27.146	161	175	190	204	219	233	248	263	277
18.8	27.292	306	321	335	350	364	379	393	408	422
18.9	27.437	452	466	481	495	510	524	539	553	568
19.0	27.582	597	612	626	641	655	670	684	699	713
19.1	27.728	742	757	771	786	801	815	830	844	859
19.2	27.873	888	902	917	931	946	961	975	990	*004
19.3	28.019	033	048	062	077	091	106	121	135	150
19.4	28.164	179	193	208	222	237	252	266	281	295
19.5	28.310	324	339	353	368	382	397	412	426	441
19.6	28.455	470	484	499	513	528	543	557	572	586
19.7	28.601	615	630	644	659	674	688	703	717	732
19.8	28.746	761	775	790	804	819	834	848	863	877
19.9	28.892	906	921	936	950	965	979	994	*008	*023
20.0	29.037	052	067	081	096	110	125	139	154	168
20.1	29.183	198	212	227	241	256	270	285	299	314
20.2	29.329	343	358	372	387	401	416	431	445	460
20.3	29.474	489	503	518	532	547	562	576	591	605
20.4	29.620	634	649	663	678	693	707	722	736	751
20.5	29.765	780	795	809	824	838	853	867	882	897
20.6	29.911	926	940	955	969	984	999	*013	*028	*042
20.7	30.057	071	086	101	115	130	144	159	173	188
20.8	30.203	217	232	246	261	275	290	305	319	334
20.9	30.348	363	377	392	407	421	436	450	465	479

SEA LEVEL DIFFERENCE BETWEEN KEY WEST AND MIAMI, FLORIDA

By

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In a previous paper (Montgomery, 1938, p. 177) it was suggested that fluctuations in the drop in sea level from Key West to Miami might indicate fluctuations in strength of the Florida Current. Both stations are located on the left side of the Straits of Florida, Key West near the entrance and Miami 130 nautical miles down the shore at the most constricted section, where it is supposed that the Florida Current runs swiftest. The acceleration of the water on approaching the constriction is accompanied by a decrease in head. In that paper it was assumed that the drop in sea level from Key West to Miami represents the total head, in other words that Key West indicates the level of the current starting practically at rest. Not knowing the absolute difference in sea level between the two stations, it was assumed to be 19 cm, the value obtained by leveling between Cedar Keys and St. Augustine. These two assumptions were in good agreement.

It was however quite fallacious to assume the water relatively motionless at the Key West section. According to Pillsbury's (1890, p. 608) measurements from the *Blake* the surface speed at the axis of the Florida Current on a section about 40 miles west of Key West is 2.24 knots, and on the Miami section it is 3.46 knots, or 115 cm sec^{-1} and 178 cm sec^{-1} respectively. From the relationship between velocity v, head h and acceleration of gravity g,

$$v_2^2 - v_1^2 = 2\ g\ (h_1 - h_2),$$

the drop in head corresponding to this velocity increase would be 9.4 cm. The expected drop from a section at Key West instead of from the *Blake* section might be three quarters of this or 7.0 cm.

Tables I and II give for Key West and Miami the monthly departures from normal of sea level reduced to normal atmospheric pressure, or the departures from normal of total head at a fixed level sufficiently low never to be exposed by the tide. These are revisions to date of the previous tables (Montgomery, 1938, Tables 2,

[1] Contribution No. 277.

4). The headings give details of the normals used. Monthly values of sea level were kindly supplied by the Director of the Coast and Geodetic Survey. All except the values for 1939 have been published by the Association d'Océanographie Physique (1939, 1940a, 1940b). The original values are in hundredths of feet, and the original values of pressure in hundredths of inches of mercury. If there is no error in the last place of the original values, the maximum error of the monthly values tabulated here is thus 0.425 cm. The mean monthly values from the two tables are plotted in Figure 4.

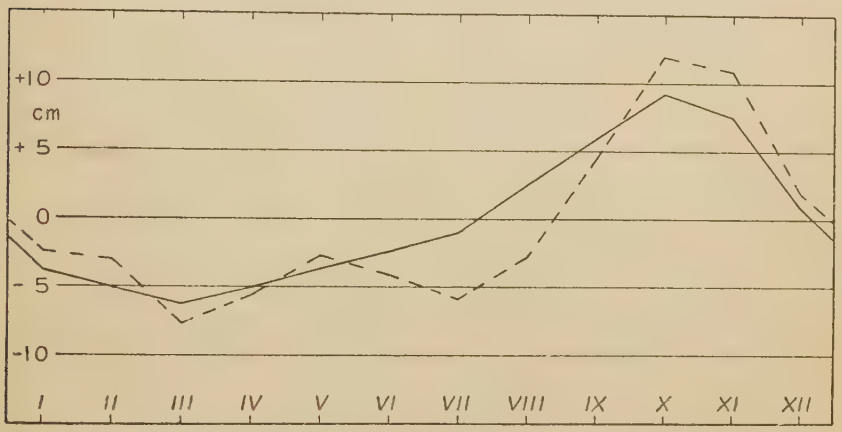

Figure 4. Annual variation of sea level reduced to normal atmospheric pressure: full line for Key West 1913–1939, broken line for Miami 1931–1939.

In March, 1934, a leveling survey was made between Miami and Key West. Key West Bench Mark 29 was 66.23 cm higher than Miami Bench Mark 6, the probable error being less than 1 cm, as stated in a communication from the Director of the Coast and Geodetic Survey.[2] Accordingly the zero on the tide staff was 42.9 cm lower at Key West than at Miami, and the normal staff reading

[2] "The probable accidental error of the first-order leveling between these two marks, computed according to the formula adopted by the International Geodetic Association in 1912, is 0.275 mm. per km. Thus, for the whole 265 kilometers between Tidal 6 (USE) and Tidal 29 the probable accidental error is 4.5 mm. The probable systematic error is of an order of size of 0.01 mm. per km. In view of the above, it would seem reasonable to assume that the probable error of the observed difference is less than one centimeter. The actual error cannot be computed, but the chances are good that it does not exceed an inch or an inch and a half."

was 3.1 cm higher at Key West. Taking into account the difference in normal atmospheric pressure at the two stations it is found that for zero departure of sea level and of pressure the head was 2.4 cm greater at Key West than at Miami.

Table III gives monthly values of the drop in head from Key West to Miami. It is to be noticed that March, 1934, is a nearly normal March, that 1934 is a nearly normal year, and that there is no secular change of the yearly values, thus giving no evidence of a vertical shift of the tide staffs from their relative positions as determined in March, 1934.

The mean value for the 8-year period of the drop in head from Key West to Miami is found to be 4.9 cm, considerably less than the value found to correspond with the increase in velocity of the Florida Current. It is possible that errors in the velocity determinations, in the leveling and in the determinations of sea level could account for this discrepancy. The drop in head of 4.9 cm would correspond to a velocity increase from 149 cm sec^{-1} to 178 cm sec^{-1}.

The variation of the individual monthly values in Table III and even the annual variation are greater than the supposed variation in increased velocity of the current from Key West to Miami. Certainly they cannot correspond at all directly to the total velocity of the current. There are even a few negative values in the table. Most of the discrepancy is probably due to the effect of local winds on the sea level at the two tidal stations.

Perhaps an extensive investigation of the wind effect would make its elimination from the sea level observations possible. At the present time however there is no justification for using the difference in sea level from Key West to Miami as an indication of the strength of the Florida Current.

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TABLE I

DEPARTURE FROM NORMAL IN CENTIMETERS OF MONTHLY SEA LEVEL AT KEY WEST REDUCED TO SEA LEVEL PRESSURE OF 30.02 INCHES

(Normal sea level is the average for 1913–1939, being staff reading of 4.95 feet; zero on staff is 13.06 feet below Bench Mark 29)

Month	*I*	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*	*IX*	*X*	*XI*	*XII*	*Year*
1913	− 5.0	− 2.6	− 5.8	− 1.4	− 1.9	+ 1.4	− 0.2	+ 6.3	+ 5.4	+ 6.7	+ 3.0	− 1.8	+ 0.3
1914	− 5.9	− 9.8	−12.0	− 8.5	+ 5.4	+ 0.6	− 4.9	+ 7.7	+ 9.7	+ 0.9	+ 1.7	+ 1.6	− 1.1
1915	− 2.2	− 2.8	− 5.2	− 5.8	−10.5	− 6.3	− 4.0	− 1.7	+ 7.3	+ 7.9	+ 5.1	− 4.0	− 1.8
1916	− 3.6	− 3.1	−10.5	− 7.8	− 2.4	− 8.2	− 1.3	+ 1.5	+ 5.6	+12.8	+10.3	− 0.4	− 0.6
1917	− 8.2	− 4.9	+ 7.6	+ 1.7	− 1.9	− 3.1	− 5.2	− 5.9	+ 8.1	+15.7	+17.9	+ 7.3	+ 2.4
1918	− 6.0	−10.2	− 6.0	− 1.0	0.0	− 5.0	− 2.3	+ 1.4	+ 3.0	+ 0.9	+ 4.8	+ 3.7	− 1.4
1919	− 6.7	−12.0	− 4.7	− 8.3	− 9.0	− 2.6	+ 0.1	+ 1.7	+ 5.7	+11.8	+ 7.6	+ 0.7	− 1.3
1920	− 8.9	− 6.9	−19.1	−12.9	− 8.8	− 1.6	+ 0.1	− 0.6	+ 3.8	+ 6.7	+ 4.3	− 1.2	− 3.8
1921	− 2.2	− 6.1	− 3.8	− 0.6	− 2.0	+ 3.5	− 0.2	+ 1.3	+ 9.9	+10.8	+ 2.6	− 0.3	+ 1.1
1922	− 6.7	− 1.0	− 2.0	− 3.6	− 4.5	− 0.7	+ 3.6	+ 2.1	+ 7.8	+ 5.6	+ 2.8	− 5.2	− 0.2
1923	− 4.5	− 5.4	− 2.9	− 2.9	− 0.9	− 2.3	− 5.0	− 4.6	+ 1.6	+ 3.5	+ 1.4	− 7.4	− 2.5
1924	− 6.0	− 4.4	− 8.4	−14.8	−11.5	− 5.2	+ 1.1	+ 4.0	+ 5.6	+ 7.6	+ 7.3	−10.1	− 2.9
1925	− 8.3	− 4.6	− 7.0	−15.1	− 4.9	− 4.5	− 3.3	+ 3.1	+ 5.2	+ 4.5	+ 9.8	+ 0.5	− 2.0
1926	−10.2	−16.6	−15.3	− 8.8	− 8.7	− 6.3	− 5.8	+ 5.2	+ 7.2	+ 9.7	+ 4.0	− 2.7	− 4.0
1927	− 0.5	− 2.2	− 1.8	− 3.4	− 4.3	− 1.9	− 4.2	+ 2.2	+ 0.1	+ 6.2	+11.5	+ 3.5	+ 0.4
1928	− 4.8	−16.0	−11.0	−12.2	− 6.3	− 2.6	+ 0.3	+ 3.6	+ 4.8	+ 9.1	+ 9.0	+ 4.9	− 1.8
1929	− 8.3	− 2.4	− 7.1	− 1.5	− 2.0	− 2.7	+ 2.4	+ 2.5	+ 3.0	+ 3.1	+ 1.0	+ 1.9	− 0.8
1930	+ 3.2	+ 1.0	− 5.5	− 3.0	− 2.0	− 4.5	− 0.8	+ 5.4	+ 2.0	+ 8.0	+ 3.7	− 4.1	+ 0.3
1931	−11.3	− 9.8	− 5.0	− 5.0	−15.2	− 8.0	− 6.3	− 4.0	+ 3.1	+ 3.2	+ 5.0	+ 0.2	− 4.4
1932	− 0.3	− 6.9	− 9.9	− 6.9	− 4.4	− 4.8	− 1.2	+ 1.5	+ 5.6	+ 4.5	+ 9.1	+11.0	− 0.2
1933	− 0.4	− 2.7	− 8.1	− 2.4	+ 0.6	+ 3.7	+ 1.7	+ 6.0	+ 8.4	+17.1	+13.2	− 0.2	+ 3.1
1934	− 1.5	− 9.1	− 6.7	− 5.3	− 9.2	− 4.8	− 2.4	− 0.6	+ 8.6	+12.2	+ 7.6	+ 5.2	− 0.5
1935	+ 3.9	− 3.9	− 0.9	− 0.9	− 4.8	− 3.2	+ 0.9	+ 4.1	+ 0.5	+16.9	+11.9	+ 1.8	+ 2.2
1936	− 2.7	− 1.8	− 8.4	− 5.4	+ 4.8	+ 4.1	+ 3.7	+ 2.8	+10.7	+16.5	+11.4	+10.2	+ 3.8
1937	+ 4.8	+ 7.6	− 5.4	− 0.1	+ 5.5	− 1.0	+ 7.4	+11.1	+11.5	+16.1	+ 9.0	+ 3.6	+ 5.8
1938	− 1.0	+ 0.1	− 4.7	− 0.9	− 0.2	+ 1.2	− 1.5	+ 7.0	+ 4.5	+10.9	+13.7	+ 3.5	+ 2.7
1939	− 0.3	− 1.6	− 0.9	− 1.8	− 0.2	− 0.1	+ 0.2	+ 4.7	+ 9.6	+18.3	+14.1	+ 1.0	+ 3.6
Means	− 3.8	− 5.1	− 6.3	− 5.1	− 3.7	− 2.4	− 1.0	+ 2.5	+ 5.9	+ 9.2	+ 7.5	+ 0.9	—

TABLE II

DEPARTURE FROM NORMAL IN CENTIMETERS OF MONTHLY SEA LEVEL AT MIAMI REDUCED TO SEA LEVEL PRESSURE OF 30.04 INCHES

(Normal sea level is the average for 1932–1939, being staff reading of 3.44 feet; zero on staff is 9.48 feet below Bench Mark 6)

Month	*I*	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*	*IX*	*X*	*XI*	*XII*	*Year*
1931						− 9.5	−11.7	− 8.4	− 1.1	+ 1.6	− 0.2	− 4.3	
1932	− 4.7	− 8.0	−11.5	− 8.6	− 7.5	− 9.1	− 6.1	− 5.5	+ 7.0	− 0.3	+ 8.8	+ 9.9	− 3.0
1933	− 3.1	− 4.2	− 8.4	− 4.2	− 2.0	+ 0.1	− 5.2	− 1.2	+ 2.4	+18.7	+15.3	− 3.2	+ 0.4
1934	− 3.3	− 8.2	− 9.5	− 6.4	− 8.9	− 5.4	− 7.3	− 4.5	+ 2.9	+12.6	+ 6.8	+ 0.7	− 2.5
1935	+ 3.6	− 1.2	− 3.5	− 1.4	− 7.4	− 4.7	− 6.4	− 5.4	− 3.8	+15.5	+17.4	+ 3.6	+ 0.5
1936	− 3.8	− 2.1	−10.5	− 8.4	+ 6.1	+ 3.8	− 5.2	− 1.4	+ 8.9	+12.2	+ 8.3	+ 8.7	+ 1.4
1937	− 1.8	+ 5.2	− 4.8	− 5.0	+ 2.2	− 6.2	+ 0.1	+ 2.3	+ 7.0	+15.8	+ 6.8	+ 0.3	+ 1.8
1938	− 2.9	− 0.8	− 7.1	− 5.7	− 3.7	− 3.6	− 7.6	− 1.2	− 2.5	+13.7	+18.0	+ 4.4	+ 0.5
1939	− 3.0	− 4.5	− 6.1	− 5.2	− 0.5	− 2.4	− 3.6	− 0.2	+13.8	+18.0	+16.8	− 3.3	+ 1.7
Means	− 2.4	− 3.0	− 7.7	− 5.6	− 2.7	− 4.1	− 5.9	− 2.8	+ 4.4	+12.0	+10.9	+ 1.9	

TABLE III

SEA LEVEL DIFFERENCE IN CENTIMETERS, KEY WEST—MIAMI, ADJUSTED FOR ATMOSPHERIC PRESSURE
(2.4 cm plus values in Table I minus values in Table II)

	I	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*	*IX*	*X*	*XI*	*XII*	*Year*
1931						+ 3.9	+ 7.8	+ 6.8	+ 6.6	+ 4.0	+ 7.6	+ 6.9	
1932	+ 6.8	+ 3.5	+ 4.0	+ 4.1	+ 5.5	+ 6.7	+ 7.3	+ 9.4	+ 1.0	+ 7.2	+ 2.7	+ 3.5	+ 5.1
1933	+ 5.1	+ 3.9	+ 2.7	+ 4.2	+ 5.0	+ 6.0	+ 9.3	+ 9.6	+ 8.4	+ 0.8	+ 0.3	+ 5.4	+ 5.1
1934	+ 4.2	+ 1.5	+ 5.2	+ 3.5	+ 2.1	+ 3.0	+ 7.3	+ 6.3	+ 8.1	+ 2.0	+ 3.2	+ 6.9	+ 4.4
1935	+ 2.7	− 0.3	+ 5.0	+ 2.9	+ 5.0	+ 3.9	+ 9.7	+11.9	+ 6.7	+ 3.8	− 3.1	+ 0.6	+ 4.1
1936	+ 3.5	+ 2.7	+ 4.5	+ 5.4	+ 1.1	+ 2.7	+11.3	+ 6.6	+ 4.2	+ 6.7	+ 5.5	+ 3.9	+ 4.8
1937	+ 9.0	+ 4.8	+ 1.8	+ 7.3	+ 5.7	+ 7.6	+ 9.7	+11.2	+ 6.9	+ 2.7	+ 4.6	+ 5.7	+ 6.4
1938	+ 4.3	+ 3.3	+ 4.8	+ 7.2	+ 5.9	+ 7.2	+ 8.5	+10.6	+ 4.4	− 0.4	− 1.9	+ 1.5	+ 4.6
1939	+ 5.1	+ 5.3	+ 7.6	+ 5.8	+ 2.7	+ 4.7	+ 6.2	+ 7.3	− 1.8	+ 2.7	− 0.3	+ 6.7	+ 4.3
Means	+ 5.1	+ 3.1	+ 4.4	+ 5.0	+ 4.1	+ 5.1	+ 8.6	+ 8.9	+ 4.9	+ 3.3	+ 2.1	+ 4.6	+ 4.9

POTENTIOMETRIC ANALYSIS OF SEA WATER
II. DETERMINATION OF TITRATION ALKALINITY

By

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Sea water is alkaline mainly due to the presence of appreciable quantities of carbonate, bicarbonate and borate ions. These react with the H^+ from the ionization of water leaving an excess of OH' present.

$$H_2O \leftrightharpoons H^+ + OH'$$

$$HCO_3' + H^+ \leftrightharpoons H_2CO_3$$

$$CO_3'' + H^+ \leftrightharpoons HCO_3'$$

$$H_2BO_3' + H^+ \leftrightharpoons H_3BO_3$$

The OH' together with the uncombined anions of weak acids may be titrated with H^+. The number of milliequivalents of H^+ needed to neutralize one liter of sea water is referred to as titration alkalinity. The major portion of the titration alkalinity is due to the effect of the carbonate and bicarbonate and only a small portion due to the borate. At the hydrogen ion concentration normally present in sea water about 92% of the total carbon dioxide is present as bicarbonate ion, about 8% as carbonate ion and less than 1% as dissolved carbon dioxide and carbonic acid.

Titration alkalinity is determined experimentally by treating 100 ml. of sea water with 5 ml. of 0.07 N HCl to convert the borate to undissociated boric acid, the carbonate and bicarbonate to carbonic acid and then by refluxing for five minutes to decompose the carbonic acid and expel the carbon dioxide. After cooling, the excess HCl is titrated with 0.025 N carbonate-free NaOH. Either methyl orange or methyl red is used as the indicator so that the titration will be stopped after the HCl has been titrated but before the boric acid ionizes and is titrated. Usually the HCl is standardized against sodium carbonate in a synthetic sea water to simulate the actual ex-

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perimental conditions as nearly as possible and thus compensate for errors of determination. Actually some difficulty is experienced in locating the exact end point of the titration using either methyl red or methyl orange as the indicator. It was thought that a potentiometric titration might eliminate this difficulty. The potentiometric

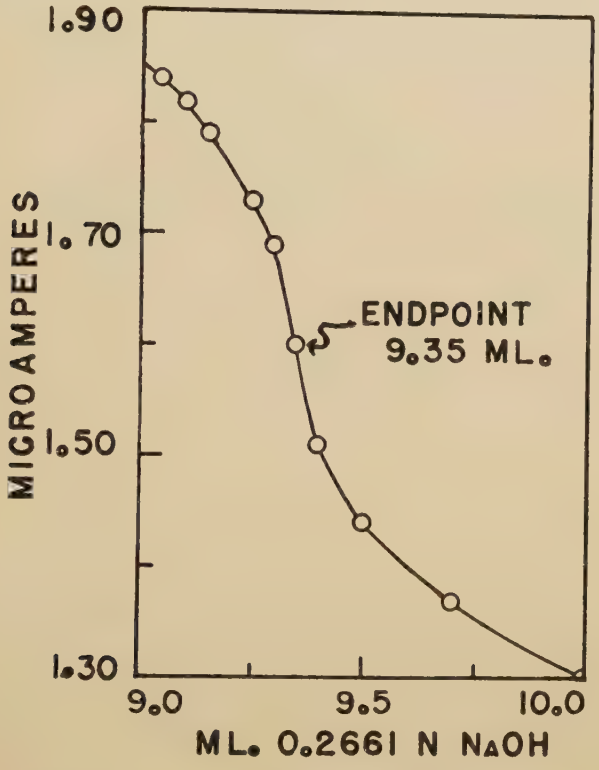

Figure 5. A Titration Alkalinity Curve by Indirect Titration.

apparatus, using bimetallic electrodes, previously described by the authors (*1*) was tried for this purpose and proved to be very satisfactory as the following description of these experiments will indicate.

Potentiometric titrations were made in a manner similar to the above described procedure, excepting no indicator was needed for determination of the end point. Twenty-five ml. of 0.01812 N HCl was added and the excess titrated with 0.02661 N base. A tungsten electrode served as the indicating electrode and natural graphite as

the reference electrode. The end point was very clearly marked as illustrated by Figure 5. The time required for titration was about the same as when indicators were used. Table I contains results of analyses of a sea water sample made in such a manner as well as analyses using indicators for the detection of the end point. In Table I are also shown results in which the titration alkalinity was determined by direct addition of 0.01812 N acid with no back titration.

TABLE I

TITRATION ALKALINITY OF A SEA WATER SAMPLE

	Back Titration with NaOH		
Methyl Orange	*Methyl Red*		*Potentiometric*
2.25			
2.15			2.07
2.18	2.07		2.01
2.21	2.03		2.04
Ave. 2.20	2.05		2.04
	Direct Titration with HCl		
			2.16
			2.17
			2.17
			2.16
		Ave.	2.17

Approximately 0.1 milliequivalent of H^+ is needed to change the H^+ concentration from the end point of methyl red to that of methyl orange, considering them to be at pH of 5 and 4 respectively. If this correction is made the results using the two different indicators agree very well. In the potentiometric back titration the most abrupt change in potential would occur as near neutrality as possible but before the H_3BO_3 would begin to ionize, i. e. at a H^+ concentration essentially at the end point of methyl red. The results confirm this conclusion. In the direct titration the end point would occur after the conversion of HCO_3' to H_2CO_3 which is near the methyl orange end point. This is also confirmed experimentally. For this reason the indirect titration should yield results more in keeping with the definition of titration alkalinity. As mentioned previously the end point with the tungsten and graphite electrodes was quite easily ascertained. In as much as this is an acid-base titration other electrodes would prove as satisfactory. Others have been used in sample acid-base titrations. In the indirect titration a buret electrode would

offer the advantage of preventing absorption of carbon dioxide from the air during titration. A glass electrode would undoubtedly also prove satisfactory. A simple hydrogen electrode could also be used, but would yield variable results. The bubbling hydrogen would wash out carbon dioxide which would not affect the sharpness of the end point but would be intermediate between the methyl red and methyl orange end points.

It is felt that the selection of the end point by potentiometric titration increases the accuracy of this titration.

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STUDIES ON MARINE BACTERIA. I. THE CULTURAL REQUIREMENTS OF HETEROTROPHIC AEROBES[1]

By

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The observations of Issatchenko (1914), Benecke (1933), Waksman (1934), ZoBell (1938) and others have established that bacteria are quite widely distributed in the sea where they probably play an important role influencing chemical, physico-chemical, geological and biological processes. However, due primarily to the widely divergent analytical procedures which have been used by various investigators studying the distribution and activities of marine bacteria, the quantitative results obtained by different workers in different parts of the world are not comparable.

The nutrient medium used to grow the bacteria has been one of the greatest variables. A large variety of nutrient ingredients in varying concentrations and combinations which yield markedly different results have been used and recommended for plate count media. Following literally Standard Methods (1933) of water analysis some workers have used fresh water to prepare their media either with or without the addition of salt, while others have used synthetic or natural sea water although, as will be pointed out below, most marine microorganisms have fairly specific salt requirements. Still other workers have regarded the bacteria of the sea as not unlike those in the soil and have treated them accordingly with discordant results. As pointed out by Lipman (1929), within relatively narrow limits the composition of the medium is an important determinant of the number and kinds of organisms found in the sea. At the present time there are almost as many kinds of nutrient media in use for estimating the marine bacterial population by plate count procedure as there are workers in the field and, as will be shown below, they yield very different results.

[1] Contribution from the Scripps Institution of Oceanography, New Series No. 124. Technical assistance in these studies was furnished by the personnel of the Work Projects Administration, Official Project No. 665-07-3-141.

The following report is concerned primarily with a systematic study of the cultural requirements of marine bacteria with special reference to the preparation of a nutrient medium for heterotrophic aerobes which will yield maximum and reproducible counts. While it is recognized that no one combination of nutrients or cultural conditions can provide for the growth of all kinds of bacteria which occur in the sea, the unquestioned value and extensive use of plating procedures for estimating the bacterial population of marine materials, for studying cultural characteristics and for obtaining pure cultures for physiological studies makes it highly desirable to have data which will permit the adoption of media which will yield maximum and reproducible counts. Moreover, such information on the nutritional requirements of marine bacteria will prove to be invaluable in studying their physiological activities and in estimating their importance as biochemical agents in the sea.

Observations during the last eight years on the occurrence and distribution of bacteria in the sea have afforded an excellent opportunity to study their cultural requirements. In analyzing sea water, bottom sediment or other marine materials it has required little additional effort to use two or more combinations of media rather than merely one and moreover, this procedure has served to check the results. Thus, over a period of years, it has been possible to compare the relative growth-promoting properties of several different media which have been proposed for the growth of marine bacteria. New formulae have been developed as indicated by the cumulative results and tried.

EXPERIMENTAL METHODS

Sea water for analysis was collected by means of a bacteriological sampler described by ZoBell and Feltham (1934) which insures exclusion of any extraneous contaminating material. Radially central portions of the cores of bottom deposits obtained with a Trask coring tube (Hough, 1939) were dissected out for analysis, using aseptic precautions in all manipulations. Except where otherwise designated, the samples were collected at places which are remote from possibilities of terrigenous contamination. Autoclaved sea water or a mixture of 75 per cent sea water with 25 per cent distilled water was used to dilute the samples, the samples being diluted sufficiently to provide for the growth of 30 to 300 colonies on the plates which were acceptable.

In the preliminary work a medium similar to that recommended by Standard Methods (1933) of water analysis was used:

Bacto-peptone	5.0 gm
Beef extract	2.0 gm
Bacto-agar	15.0 gm
Sea water	1000.0 ml

This will be referred to as Formula 1 and has been used as the standard for comparing many other media. The sea water used in the preparation of the media, unless otherwise stated, was collected and aged for several weeks in 5 gallon glass bottles to insure uniformity in the composition.* The reaction of Formula 1 is pH 7.6 without adjustment after sterilizing at 120° C. for 20 minutes. Special care was taken to cool the nutrient agar to 42° C. before pouring into the inoculated plates because, as has been pointed out by ZoBell and Conn (1940), most marine bacteria are thermo-sensitive, many of them being injured by even short exposure to temperatures exceding 42° C. The inoculated plates were incubated at 22° C., it having been shown that prolonged exposure at higher temperatures is lethal to some marine bacteria. The colonies which developed on the plates after five to ten days were counted, using a 3.5X engraver's lens and a Stewart counting chamber.

SALINITY REQUIREMENTS

Fresh water, either with or without the addition of salts, has been quite commonly used for estimating the bacterial population of marine materials. Lloyd (1930), Burke (1934) and others report that marine bacteria grow equally well in either fresh water or sea water media. Conversely, Berkeley (1919), Korinek (1926) and Lipman (1926) maintain that the mere dilution of sea water materially reduces the number of marine bacteria which will grow. According to Korinek (1927) marine bacteria can be distinguished from non-marine forms upon a basis of their specific salt tolerance.

*Matudaira (1939) and others have noted a marked difference in the growth-promoting properties of sea water collected at different depths, stations and dates on the growth of diatoms which is attributed to something organic in nature. Similarly we have noted that bacteria behave differently in media prepared with sea water collected from different stations. These differences in the growth-promoting properties of sea water disappear within a few weeks when the sea water is stored or "aged" at room temperature presumably because the organic fractions which are responsible for the differences are oxidized by the increased bacterial activity which accompanies the storage of sea water in glass bottles (ZoBell and Anderson, 1936a).

The validity of these antithetical views was tested by preparing nutrient media which were similar in all respects except salt content. For this purpose, Formula 1 was used with the sea water diluted with distilled water to give various concentrations. These media were inoculated with appropriately diluted samples of raw sea water and their relative growth-promoting properties determined. Table I gives the protocol of a representative experiment. To facilitate the comparison of results the "growth index" for each medium is recorded. The growth index is the expression of the average plate counts as ratios on a basis of the plate counts on the standard medium being 100.

TABLE I

Relative Numbers of Bacteria in a Sample of Raw Sea Water which Formed Colonies in Nutrient Media Prepared with Different Concentrations of Sea Water

Per cent sea water	100	75	50	25	10	5	0
Per cent distilled water	0	25	50	75	90	95	100
Bacteria in sample 9A	218	196	139	87	38	24	13
Bacteria in sample 9B	231	204	143	82	35	28	21
Average number of bacteria	224	200	141	84	36	26	17
Growth index	**100**	89	63	41	16	12	8

Over thirty samples of sea water and nearly as many samples of marine bottom deposits collected from various stations off the coast of Southern California have been analyzed in this way. Without exception appreciably more bacteria grew in the media prepared with sea water than in corresponding fresh water media. Only 55 to 65 per cent as many colonies appeared on plates of media prepared with sea water diluted one half with fresh water, and in fresh water media only 5 to 20 per cent as many bacteria developed as in that prepared with undiluted sea water. Similar results were obtained regardless of whether tap water, distilled water or glass re-distilled water was used, thus ruling out the possibility of the fresh water carrying any toxic agent. From the data it is quite apparent that sea water and not distilled water or tap water should be used in the preparation of nutrient media for marine bacteria.

In the experimental procedure outlined above 1.0 ml. of raw sea water or mud diluted with sea water was used to inoculate approximately 10 ml. of nutrient agar. It follows, then, that upon the inoculation of the fresh water medium in which no sea water was used in its preparation, there is around 10 per cent of sea water in the medium, and the other media will actually contain a somewhat larger percentage of sea water than that designated in the table. Sea water

can be almost completely excluded from the fresh water media by the use of fresh water for diluting mud and other marine materials containing a rich microflora, but another complication is introduced: the viability of marine bacteria is rapidly weakened by such treatment. In a few experiments in which fresh water dilution water was used there were only 3 to 5 per cent as many bacteria developed in the fresh water medium as in that prepared with sea water.

Bacteria in bottom deposits are slightly less sensitive to salinity changes than are those which occur in water as shown by the composite data in Table II. Those obtained from terrestrially polluted

TABLE II

Relative Numbers of Bacteria from Marine and Terrestrial Sources which Developed in Nutrient Media Prepared with Different Concentrations of Sea Water

	Samples	*Per cent of sea water in medium*					
Source of Inocula	*Analyzed*	100	75	50	25	10	0
		Average growth index					
Raw sea water	31	**100**	93	65	38	17	9
Marine mud	18	**100**	89	61	46	28	19
Mission Bay mud	4	**100**	107	117	142	156	106
San Diego Bay mud	3	**100**	108	131	142	139	114
Mission Bay water	5	**100**	103	115	108	118	97
Sewage	13	13	27	51	79	106	**100**
Tap water	8	4	10	28	58	105	**100**
Soil (near sea)	7	48	56	79	90	97	**100**
Soil (inland)	6	15	23	40	66	89	**100**
Mouth microflora	5	9	15	41	78	93	**100**

bays (Mission Bay and San Diego Bay) seem to grow almost equally well in fresh water or sea water nutrient media, or at least there are about as many bacteria capable of growing in fresh water as in sea water media. The growth of terrigenous or fresh water bacteria is inhibited by sea water. Several samples of sewage, tap water, soil and microflora from the buccal cavity of man were plated with media containing different concentrations of sea water and the results are summarized in Table II. The samples of microflora from the buccal cavity were obtained by having several laboratory workers rinse their mouths with sterile saline and pooling the washings.

It is noteworthy that soil bacteria collected from inland valleys were much more sensitive to sea water than those in soil samples collected on the Scripps Institution grounds adjacent to the sea. How-

ever, this is not surprising because ZoBell and Mathews (1936) have shown that from 70 to 95 per cent of the bacterial flora being transported by off-sea breezes are of marine origin and that marine bacteria occur in the atmosphere 30 miles inland. Most of the bacteria which are transported inland from their marine environment to which they are indigenous soon perish; more resistant ones may survive for a time and a few probably have become acclimatized to the soil with attendant changes in their properties (Burke and Baird, 1931; Burke, 1934). Consequently, one would expect to find more marine-like bacteria in the soil near the sea than farther inland. According to ZoBell and Mathews (1936) the microflora transported by off-sea breezes is predominately terrestrial in character, fewer than half of the bacteria being able to grow in sea water media. Thus, the wind plays an important role in the interchange of bacteria between the land and sea and helps to account for the fact that 5 to 20 per cent of the bacteria in the sea are capable of growing in fresh water media and a like proportion of the bacteria in fresh water or terrestrial materials are capable of growing in sea water media. However, as will be elaborated elsewhere, certain bacteria are euryhaline.

Although a large proportion of the bacteria in marine materials are definitely stenohaline upon initial isolation, they rapidly lose their saline specificity following laboratory cultivation. After a few weeks most cultures which initially would grow only in sea water media grow equally well in either sea water or fresh water media (ZoBell and Michener, 1938).

The specific salt requirements of bacteria from oceanic environments as compared with those from brackish water, polluted bays, fresh water or terrestrial sources may account for many of the contradictory opinions expressed in the literature concerning the characteristics of marine bacteria. It is astonishing to note how many kinds of water bacteriologists have designated "sea water" without any qualifying statements concerning the salinity, isolation from oceanic circulation or other properties. Since much of the early work was done on samples obtained from seas or surface water having a low salinity, bays, harbors, estuaries and other coastal waters which are subject to varying degrees of dilution with freshwater and terrigenous contamination, one does not know if the workers were dealing with either marine bacteria or sea water.

An attempt has been made to ascertain just what there is in sea water which makes it indispensable for the initial growth of most marine bacteria, but the complete explanation has not yet been found. As shown by the data in Table III, the addition of 3.0 per cent

TABLE III

Relative Number of Bacteria in 8 Samples of Raw Sea Water and 6 of Marine Mud which Developed in Comparable Media Prepared with Various Salt Solutions

	Natural sea water	*3.0% sodium chloride solution*	*Artificial sea water*	*Isotonic sea salt solution*	*Distilled water*
			Average growth index		
Raw sea water	100	56	77	89	7
Marine mud	100	61	74	94	16

sodium chloride to fresh water nutrient medium to render it isotonic with sea water materially improves results, but such a medium is not as good as sea water for the growth of marine bacteria. Synthetic or artificial sea water prepared with several salts including a trace of ferric phosphate in the hypothetical combinations in which they occur in sea water (Lyman and Fleming, 1940) is better than the sodium chloride solution, but it fails to provide for the growth of as many bacteria as natural sea water. There seems to be something in sea water besides its mineral constituents which is essential for the growth of marine bacteria because not even isotonic concentrations of sea salt were as good as natural sea water (Table III). The sea salt was prepared by evaporating sea water in vacuo and then adding distilled water to prepare nutrient media. Allen (1913) found that certain diatoms would not grow in synthetic media unless a little sea water was added, an observation often made on other organisms.

Contrary to popular conception, marine bacteria do not tolerate high concentrations of salts much better than they tolerate hypotonicity. This is illustrated by the results summarized in Table IV,

TABLE IV

Relative Numbers of Bacteria in 12 Samples of Raw Sea Water which Developed in Nutrient Media Prepared with Sea Water to which Different Salts Were Added

			Per cent of salt added			
Salts added to sea water	0	1.5	3.0	6.0	12.0	18.0
			Average growth index			
Sodium chloride	100	96	88	41	12	3
Artificial sea water	100	90	72			
Sea salt	100	81	49			

which were obtained by inoculating media prepared with sea water to which different concentrations of sodium chloride, artificial sea water constituents or evaporated sea salt were added. Surprisingly enough, sea water rendered hypertonic with evaporated sea salt or artificial sea water constituents was more toxic than that rendered hypertonic by the addition of sodium chloride. This is probably because marine bacteria are more sensitive to large concentrations of certain specific salts found in sea water than they are to hypertonicity. The substitution of potassium chloride and sodium sulphate for sodium chloride revealed that sulphate and potassium are tolerated in concentrations two to three times higher than that in which they occur in sea water. Similar procedures showed that calcium and magnesium occur in sea water in concentrations which are near the limits of tolerance, double this amount inhibiting the growth of many marine bacteria. According to Korinek (1926, 1932) fresh water bacteria are much more resistant to changes in osmotic pressure than are marine bacteria. This was also recognized by Coupin (1915), Lipman (1926), Berkeley (1919) and others.

Further studies on the effect of diluted and concentrated sea water upon bacterial multiplication established that while marine bacteria are sensitive to salinity changes, they grow equally well in nutrient sea water throughout a salinity of 30 to 40‰. The growth of about ten per cent of them is inhibited if sea water is diluted until the salinity is 25‰ or if it is concentrated to a salinity of 45‰. Since the salinity of sea water the world over is well within these limits, comparable results should be obtained as far as salinity is concerned, regardless of where sea water is collected for the preparation of bacteriological media.

EFFECT OF NITRATE

Probably due primarily to the work of Gran (1902) and Drew (1911, 1914) on the abundance in the sea of denitrifying or nitrate-reducing bacteria whose demonstration requires the use of nitrate in the medium, many subsequent workers have continued to add nitrate in varying amounts to nutrient media used for the cultivation of marine bacteria. Though differing otherwise in composition, the media used by Berkeley (1919), Lipman (1926), Bavendamm (1931), Gee (1932) and many other leading marine microbiologists all have nitrate in common. The more or less general adoption of nitrate as a standard constituent of nutrient media recommended its use in our preliminary surveys on marine bacteria (ZoBell and Feltham, 1934) but controlled experiments with media without nitrate failed to confirm its merits.

The addition of 0.005 to 0.10 per cent of potassium nitrate to Formula 1 did not increase the plate counts and there was some evidence of the higher concentrations being inimical. This is illustrated by the results which are summarized in Table V. Similarly,

TABLE V

Effect of Different Concentrations of Potassium Nitrate on the Plate Counts of Marine Bacteria. The Growth Indices Rather than the Absolute Plate Counts Are Given

Sample	*Number of Samples*	*Concentration of Potassium Nitrate*				
		0	0.005	0.01	0.05	0.10
		Average Growth Index				
Sea water	6	**100**	102	95	102	87
Sediment	5	**100**	99	101	96	84

the nitrate neither increased the size of the colonies nor the number of kinds of bacteria which developed. Sodium nitrate was not unlike potassium nitrate in its effect upon the growth and multiplication of marine bacteria. In the course of the investigations nitrate was added to several other combinations of nutrients which were tried but in no case did it prove to be beneficial.

According to Gran (1902), certain marine bacteria can use nitrate as the sole source of nitrogen. It is now recognized that many bacteria are endowed with this ability, but the author is aware of no bacterium for which nitrate is indispensable for its nitrogen nutrition. Nitrate-reducing, denitrifying or nitrate-assimilating bacteria have been found to grow just as well in the absence of nitrate as in its presence. This generalization applies to nearly a hundred pure cultures which have been tested and it substantiates the plate count observations that the addition of nitrate to nutrient media does not enhance the growth of marine bacteria. Moreover, just as many nitrate-reducing or denitrifying organisms are found on plates of nutrient media without nitrate as on plates of similar media containing 0.05 per cent potassium nitrate. Therefore, it would seem that there is no reason for adding nitrate to the medium unless the latter is to be used for a specific purpose for which nitrate is required; for example, testing bacteria for their ability to reduce nitrates.

PHOSPHATE

Lea and Nichols (1936) believe that a lack of sufficient phosphate in natural waters not infrequently limits the growth of bacteria, but

the work of Waksman and Carey (1935) and Renn (1937) indicates that it is doubtful if phosphate limits the growth of bacteria in the sea. Nevertheless, it may be desirable to supplement the phosphate content of sea water used in the preparation of nutrient media where, besides supplying the phosphorus requirements of bacteria, it might also function as a buffer and in other ways. Certain workers (Reuszer, 1933; Lipman, 1929; Bedford, 1933) recommend the addition of 0.005 to 0.10 per cent of potassium phosphate to nutrient sea water media while others make no provision for supplementing the phosphorus content. The effect of this variance in technique on the growth of marine bacteria has been investigated.

Dibasic potassium phosphate in concentrations ranging from 0.005 to 0.1 per cent were added to Formula 1. The reaction of each medium was adjusted to pH 7.6 after autoclave sterilization. The media were inoculated with 1.0 ml of appropriately diluted samples of raw sea water or marine mud. Table VI shows the relative numbers of colonies which developed on each medium. Included in Table VI are also the results obtained with and without the use of dibasic potassium phosphate in Reuszer's (1933) and PPP media:

Peptone	1.0 gm	Proteose peptone	2.0 gm
Glucose	1.0 gm	Bacto peptone	3.0 gm
K_2HPO_4	0.05 gm	Beef extract	2.0 gm
Agar	15.0 gm	Agar	15.0 gm
Sea Water	1000.0 ml	Sea Water	1000.0 ml

The three nutrient media were not used simultaneously so the data in Table VI should not be misconstrued as comparing their relative

TABLE VI

Effect of Dibasic Potassium Phosphate on the Growth of Marine Bacteria

Nutrient Medium	*Samples Tested*	*Concentration of K_2HPO_4*				
		0	0.005	0.010	0.025	0.10
		Average growth index				
Formula 1	6	100	104	98	103	91
Reuszer's	6	100	105	102	101	84
PPP	8	100	97	104	98	96

merits. The data show how the addition of phosphate influenced the growth of bacteria in each medium. It will be observed that within the limits of experimental error bacterial development was indifferent to the presence of the added phosphate.

There is some evidence of the lower concentrations (0.005 to 0.01 per cent) of dibasic potassium phosphate improving the media slightly but the results are not conclusive. Definitely, it is not inimical to the growth of marine bacteria unless used in nutrient sea water media in concentrations exceeding 0.025 per cent. Since marine bacteria are quite sensitive to changes in the hydrogen-ion concentration, the use of phosphate might be indicated in media containing little or no peptone or other good buffers.

The apparent inhibitory effect of 0.1 per cent potassium phosphate is probably not due to any direct effect of the salt on the bacteria because, as shown by Mallman and Gallo (1931) the growth and metabolism of many bacteria is improved by the use of this much sodium or potassium phosphate in nutrient media. When added to sea water the phosphate tends to precipitate the calcium, iron and other salts from solution, and in large concentrations tends to materially affect the mineral composition of sea water. This complication can be largely obviated without diminishing the theoretical or real benefit of additional phosphate by the use of one which is relatively insoluble, such as tricalcium or ferric phosphate. Since iron salts have proved to be highly beneficial, the use of ferric phosphate is recommended.

IRON AND OTHER MINERALS

While testing the tolerance of marine bacteria for iron salts, it was observed that the addition of small quantities of either ferrous or ferric salts to nutrient sea water media materially increased the plate counts. Although sea water itself probably contains enough iron (Thompson and Robinson, 1932; Cooper, 1935) to support an extensive bacterial population and the peptone, beef extract and other media constituents also contain more iron than that required for the cell substance of the bacteria which grow in such media, the iron might not be in the right form or concentration to provide for optimal conditions. Evidence will be presented elsewhere which suggests that iron like aluminum compounds precipitate from solution or otherwise inactive toxic agents such as silver or copper, for example, which occur in sea water and which may have an unfavorable oligodynamic action on microorganisms. It is also probable, as suggested by Baudisch (1932), that iron in the proper form and concentration acts as a biocatalyst and otherwise stimulates bacterial growth. Allen (1913), Gran (1931) and others have observed that the addition of iron to sea water cultures stimulates the growth of diatoms. The compilation of culture media by Levine and Schoenlein (1930) shows that iron salts are widely used to promote the growth of fastidious microorganisms.

Preliminary experiments revealed that from a viewpoint of their availability and effect on bacterial development, ferric chloride, ferrous sulphate, ferric citrate and ferric phosphate were the best iron salts to use in nutrient sea water media. Table VII summarizes the

TABLE VII

Relative Number of Bacterial Colonies which Developed on Nutrient Sea Water Agar with Different Concentrations of Iron. The Average Plate Counts of Six Samples of Sea Water are Given Expressed as Ratios upon a Basis of the Plate Count on the Control Medium Sans Additional Iron Being 100

Salt used	*Molecular Weight*	*Concentration in per cent* 0	0.001	0.01	0.10
			Growth Index		
Ferric citrate	356.58	100	143	163	107
$FeCl_3 \cdot 6\ H_2O$	270.31		132	154	82
$FeSO_4 \cdot 7\ H_2O$	278.02		134	141	90
$FePO_4 \cdot 4\ H_2O$	222.93		149	178	126

results obtained when using different concentrations of these salts in a medium prepared with 0.3 per cent Bacto-peptone, 0.2 per cent proteose peptone, 0.2 per cent beef extract and 1.5 per cent agar in sea water. The reaction of each medium was adjusted to pH 7.6 with the addition of N/1 NaOH. The media were inoculated in duplicate with six different samples of sea water and the colonies were counted after 7 days incubation at 22° C. The results are reported as ratios upon a basis of the plate count on the control medium to which no iron was added being 100.

It will be observed that the enrichment of the medium with 0.001 to 0.01 per cent of the iron salts increased the plate counts 32 to 78 per cent. One-tenth per cent of ferric citrate or ferric phosphate was less stimulating and this concentration of ferric chloride or ferrous sulphate was slightly toxic. Not only are the plate counts significantly higher in media enriched with favorable concentrations of iron salts; the colonies are larger and more kinds of bacteria develop on iron-enriched media than on control media to which no iron is added.

Further experiments with various nutrient combinations and different iron salts show that ferric salts are no better than ferrous salts for stimulating the growth of marine bacteria in nutrient sea water media. It has been noted that regardless of whether ferrous or ferric iron is added to the medium, both forms are present following its

sterilization and inoculation, the concentration of each depending upon the oxidation-reduction potential of the medium, its pH and the initial concentration of iron added. Being highly ionized and acidic, high concentrations of sulphates or chlorides of iron are more toxic than similar amounts of the poorly soluble and nearly neutral phosphates or citrates of iron. Moreover, since the addition of even low concentrations of iron chlorides or sulphates precipitate certain constituents from sea water necessitating the filtration of the media and making it difficult to adjust the pH recommends the use of citrates or phosphates of iron which do not present these difficulties. Statistically different nutrient combinations have yielded somewhat larger plate counts when ferric citrate or ferric phosphate has been used than when ferrous sulphate or ferric chloride has been used in the optimum concentration. It is much easier to prepare clear media of the proper pH when the iron is added as the citrate or phosphate rather than as the chloride or sulphate.

Although there are many species of marine bacteria which can utilize citrate as a sole source of carbon and energy, the addition of citrate to peptone medium does not increase the number of colonies which develop from sea water or sediment samples. Therefore, the beneficial effects from the addition of ferric citrate are to be ascribed to the iron and not the citrate. However, as pointed out above, additional phosphate in nutrient media might serve a useful function which would indicate the use of ferric phosphate as a source of iron in preference to ferric citrate. Further experiments with the use of these two iron salts alone and together in different concentrations in different combinations of nutrients show that nothing is gained by using both ferric citrate and ferric phosphate. There was little difference observed in plate counts, colony size or diversity of species, regardless of whether ferric citrate or ferric phosphate was added to the media, but under most conditions the use of either one increased appreciably the plate count on nutrient sea water agar.

Since both ferric citrate and ferric phosphate are only slightly soluble in sea water, an excess of either is not toxic. Almost equally good results were obtained from the use of any concentration of either salt between 0.001 and 0.025 per cent. For convenience and simplicity, 0.01 per cent, 0.1 gram per liter, has been selected for routine use. In no combination of nutrient sea water medium tried, except in acidic ones, has this concentration of ferric phosphate or ferric citrate exhibited any inimical effects, and in neutral or slightly alkaline media it has increased the plate counts by 20 to 80 per cent or more. The addition of 0.01 per cent of either salt to 0.5 per cent peptone sea water agar gives a clear medium without filtration having a re-

action of pH 7.5 to 7.6. The average plate counts of nearly a hundred samples of sea water and marine mud are between 3 and 4 per cent higher when ferric phosphate was used than when ferric citrate was used. Both yield equally reproducible results. This slight statistical advantage of ferric phosphate, together with the theoretical and possibly practical results from the phosphate enrichment of nutrient sea water media, recommends its use. Studies on a large number of pure cultures of marine bacteria to be reported elsewhere showed that the addition of ferric phosphate to various sea water media accelerated the growth and metabolism of the bacteria.

In testing the growth-promoting properties of iron in nutrient media of different pH values it was noted that iron is less stimulating as the pH decreases and is more beneficial with increasing pH, although as will be amplified below, maximum plate counts are obtained on media having a reaction of pH 7.5 to 7.8, irrespective of the presence of iron. This is illustrated by the data summarized in Table VIII which shows the average number of bacteria in twelve

TABLE VIII

Relative Number of Bacteria which Grew on Plates of Nutrient Sea Water Agar of Different pH Values with and without the Addition of 0.01 per cent Ferric Phosphate Expressed as Ratios on a Basis of the Number of Colonies which Developed on the Medium Sans Iron at pH 7.5–7.8 Being 100. The Increase or Decrease in the Plate Count Caused by the Iron in Media of Different pH Values Is also Given Expressed as per cent

pH of medium	5.8–6.0	6.4–6.6	7.0–7.2	7.5–7.8	8.1–8.3	8.4–8.7
Growth index without iron	38	53	71	**100**	82	26
Growth index with iron	21	54	99	158	142	58
Percentage difference with iron	−44.8	+1.9	+39.4	+58.0	+73.2	+123.0

samples of sea water which grew on plates of nutrient sea water agar of different pH values, with and without the addition of 0.01 per cent ferric phosphate, the plate counts being expressed as ratios on a basis of the number of colonies which developed on the medium sans iron at pH 7.5 to 7.8 being 100. Further analysis of these data reveal that whereas 0.01 ferric phosphate reduced the plate count by 44.8 per cent in media at pH 5.8 to 6.0, it augmented the plate count in less acidic media from 1.9 to 123.0 per cent, the greatest stimulation occurring in the more alkaline media.

Lower concentrations of iron were less toxic in acidic media which suggests that the increased solubility of iron in acidic media may

account for its increasing toxicity with decreasing pH. In view of the fact that 0.01 per cent of any soluble iron salt is from ten to a hundred times as much as Hotchkiss (1923) or Elvehjem (1931) have found to be tolerated by microorganisms, it is believed that marine bacteria seem to tolerate large concentrations of iron salts in sea water media only because virtually all of the iron is precipitated from solution by the phosphates, carbonates and other constituents of sea water. Additional studies will be required to elucidate the mechanism of the beneficial effects of iron salts in nutrient sea water media, but for the practical purpose of cultivating marine bacteria the use of concentrations which are theoretically toxic (0.001 to 0.01 per cent of ferric phosphate) are indicated. The foregoing observations emphasize that consistent or reproducible results are contingent more upon the pH of the sea water media than the amount of iron added.

When used in nutrient sea water media to which no iron was added, from 0.001 to 0.01 per cent of aluminum chloride stimulated the growth of marine bacteria, but not to the same extent as similar concentrations of iron salts. There was no synergetic action of aluminum and iron, the two used together producing results which were no better than when iron only was added. Low concentrations of manganese salts were also beneficial in media to which no iron salts were added, but less beneficial than either iron or aluminum. Manganese failed to fortify media which were enriched with iron or aluminum. Sea water probably contains more than enough aluminum and manganese to provide for the growth requirements of marine bacteria; the beneficial effect from the use of additional amounts of these elements probably being attributable to a catalytic or regulative function for which purpose iron is superior. According to Harvey (1939) small quantities of manganese stimulate the growth of marine diatoms.

Zinc is an indispensable nutrient for many microorganisms. It is always present in sea water although in some places only in traces (Thompson and Robinson, 1932). Since the enrichment of sea water media with varying concentrations of zinc salts failed to stimulate bacterial multiplication, it can be assumed that there is enough zinc in sea water to provide for the growth requirements of those bacteria which need it.

A lack of silica is believed by some workers to limit the growth of diatoms at certain places in the sea. It is still indeterminate if silica is required for the growth of marine bacteria but no beneficial effects followed the addition of small amounts of sodium silicate to nutrient media.

HYDROGEN ION CONCENTRATION

The pH of surface sea water ranges from 7.8 to 8.8 and decreases with depth. Very little is known concerning the hydrogen ion concentration of marine sediments but the pH of those which have been investigated at the S.I.O. ranged from 5.3 to 9.8. The pH of most of the samples of sea water whose bacteriological analysis is discussed in this paper ranged from 8.0 to 8.3; the sediments from pH 7.4 to 8.5.

If it can be assumed that the environment to which bacteria are indigenous provides conditions which are more or less optimal for

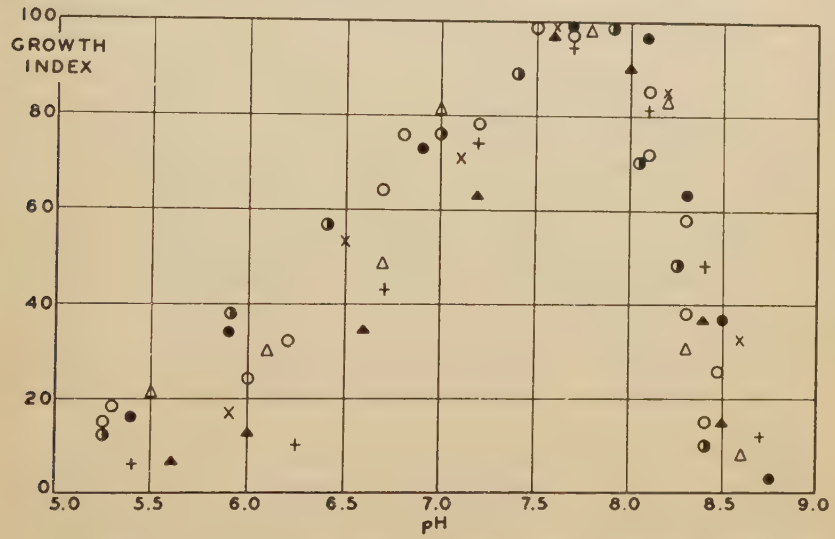

Figure 6. Effect of pH on the multiplication of marine bacteria. Circles show growth indices of bacteria in media not enriched with iron and triangles and crosses show growth indices of bacteria in media enriched with iron.

the bacteria, one would expect marine bacteria to grow best at pH 8.0 to 8.3. However, early in the work it was noted that when cultivated under laboratory conditions this pH is near the upper limit tolerated by the bacteria from the sea, the maximum development occurring in nutrient sea water media at pH 7.5 to 7.8. This is illustrated by the data summarized in Figure 6 on which the average results of seven different experiments are recorded. The pH of the media was determined with glass electrodes using a Beckman pH meter.

The pH of the nutrient sea water media is readily reduced to the desired level by the addition of HCl and it does not change much with autoclave sterilization, but considerable difficulty is encountered

in maintaining sea water media at a high pH without materially altering its composition due to the pronounced effect of carbon dioxide on the hydrogen ion concentration of sea water and other peculiarities of its buffer mechanism (Thompson and Robinson, 1932). By depressing the ionization of hydroxyl ions the addition of a little alkali to sea water medium may result in a decrease rather than an increase in pH. The pH is increased by the addition of more alkali but in the meantime much of the calcium, magnesium, iron and perhaps other cations are precipitated from solution along with the carbonates, phosphates and maybe other anions. The precipitation of these constituents, some of which may be essential for the growth of bacteria, is accentuated by heat as during the autoclave sterilization of the media particularly if the pH exceeds 7.8. Therefore the data presented in Figure 6 do not prove that reactions alkaline to pH 8.0 are inimical to marine bacteria because the composition of the sea water has been altered in other respects. However, the data show that it is best to adjust the pH of nutrient sea water media at 7.5 to 7.8. Experiments with sea water broth sterilized by Berkefeld filtration show that most marine bacteria multiply readily in media as alkaline as pH 8.5 and some tolerate a reaction of pH 10.0–10.3 although the best growth occurred in tubes of broth having a pH around 7.6.

Our Medium 2216 consisting of 0.5 per cent Bacto-peptone, 0.01 per cent ferric phosphate and 1.5 per cent agar in sea water has a terminal reaction of pH 7.5 to 7.6 following sterilization. Unlike more basic media which take up carbon dioxide from the atmosphere and thus slowly become more acid in reaction particularly after being poured in plates, this medium undergoes very little change in reaction after it is prepared. Its buffer capacity at pH 7.5 to 7.6 is adequate to prevent much change in the reaction even from the growth of bacteria. Many marine bacteria liberate ammonium from proteinaceous substrates which would have a tendency to increase the pH but ostensibly such a change is counterbalanced by the carbon dioxide which is produced by the respiring bacteria together with that which is taken up by the medium from the atmosphere.

SUCCESSIVE DILUTION METHOD vs. PLATE COUNTS

The recorded observations of Domogalla (1932), Waksman, et al. (1933), Bere (1933) and others that plate counts detect only a small percentage of the bacteria present in water suggested the advisability of examining the possibilities of applying the successive dilution method with liquid media to the enumeration of the marine bacterial

population. Theoretically, liquid medium is more suitable for the growth of water bacteria than solid medium and moreover, as elaborated by Butterfield (1933), the former is used in tubes which are easily manipulated aseptically and it makes some provisions for the simultaneous tests on the physiological characteristics of the bacteria. According to Butkewitsch (1932) the inoculation of tubes of liquid media with successive dilutions of material from the Barents Sea demonstrated the presence of from ten to a hundred times as many bacteria as plate counts on solid media.

Parallel analyses on a large number of samples of sea water and bottom sediments were made, using nutrient agar for plate counts and the successive dilution method with the identical medium without agar in test tubes. Plates were poured in duplicate, care being exercised to cool the nutrient agar to 42° C. before pouring into the plates inoculated with bacteria. The colonies which developed were counted after incubating the plates two weeks at 22° C. Following the procedure prescribed by Ziegler and Halvorson (1935) 1.0 ml. portions of each of three to five or more successive dilutions of the material differing by log one were used to inoculate the liquid medium, ten tubes being inoculated with each dilution. Thus, a minimum of thirty to fifty or more tubes were used for each sample analyzed, the number of dilutions required depending upon one's ability to estimate beforehand the order of magnitude of the bacterial population of the material being examined. With water from the open sea, which rarely contains more than a few hundred bacteria per ml. it is a matter of ease to select three effective dilutions, but with sedimentary material which may contain from less than a hundred bacteria to several million per ml., it is sometimes necessary to use eight or nine dilutions in order to insure the inoculation of the medium with three effective dilutions. Table IX shows the number of tubes inoculated with each dilution in which growth occurred within two weeks at 22° C. and the most probable number of bacteria indicated thereby according to the probability table of Halvorson and Ziegler (1933). The average number of bacteria indicated by plate counts on the same sample is also given. In order to simplify the comparison of results obtained by the two methods, only the significant figures are recorded.

A total of 38 samples were analyzed in this way, the dilution method demonstrating more bacteria in 23 of them and the plate counts being higher in the other 15 samples. The ratio of the dilution method counts to the plate counts ranged from 0.51 to 1.89; the average for the 38 samples being 1.12. In other words, the composite results show the dilution method counts to be an average of

TABLE IX

MOST PROBABLE NUMBER OF BACTERIA IN SAMPLES OF MARINE MATERIAL INDICATED BY THE SUCCESSIVE DILUTION METHOD AND THE PLATE COUNT OF THE SAME SAMPLE AND DILUTION

Sample No.	*Positive tubes in dilution*			*Successive dilution method count*	*Corresponding plate count*	*Ratio of dilution method to plate count*
	n_{10}	$n_{1.0}$	$n_{0.1}$			
1234	10	8	4	2210	1230	1.84
1235	10	10	2	3490	2120	1.64
1237	10	6	1	933	1590	0.59
1238	10	8	0	1300	1740	0.75
1314	10	7	0	1010	820	1.23
1315	10	4	2	700	370	1.89
1318	9	6	0	381	750	0.51
1319	9	8	3	499	580	0.86
1412	10	7	1	1160	690	1.68
1413	7	4	3	208	320	0.65
1432	7	4	0	171	260	0.66
1433	8	4	0	217	260	0.83
1443	10	6	0	792	630	1.26
1444	10	8	1	1500	830	1.81

12 per cent higher than the plate counts. Similarly, Butterfield (1933) found that when enough tubes were used under standardized conditions, plate counts are not markedly different from dilution method estimates of water bacteria. Ziegler and Halvorson (1935) note good agreement between plate counts and dilution method counts with some organisms but not with others, probably due to the predilection of some organisms for liquid media and others for solid media. It is believed that in most reported cases in which many times more water bacteria were demonstrated in liquid media by the dilution method than on plates of solid media, the investigators either (a) failed to use enough tubes or enough effective dilutions to get an accurate estimate of the bacterial population, (b) they were dealing with highly specialized organisms, (c) the solid medium was deficient in some property, or (d) the solid media were poured so hot that many of the thermosensitive bacteria were killed. According to Drew (1912), marine bacteria are very sensitive to temperatures as high as 40° C. and many are killed at 45° C. ZoBell and Conn (1940) report that pouring nutrient agar at temperatures exceeding 50° C. kills a large proportion of the bacteria from the sea. Working under field conditions or in an improvised laboratory on a rolling boat at sea, it is not always expeditious to cool the media to 42° C., although such treatment is prerequisite to reliable results.

During the last eight years, several different combinations of nutrients have been used to compare plate counts and successive dilution method counts. Whenever enough tubes of nearly any kind of medium tried have been inoculated with enough effective dilutions of inocula to yield reasonably reproducible results, this procedure has demonstrated the presence of very few, if any, more bacteria than equally carefully conducted plate counts. This generalization applies to the mixed heterotrophic bacterial population of sea water and marine sediments and does not apply to a few certain specific types of bacteria such as the nitrifiers, for example, which do not form colonies readily on solid media immediately following initial isolation.

In order to compare the reproducibility of results with plate counts and that of dilution method counts, a series of ten water samples were analyzed in duplicate by each method. Each of two plates of nutrient agar were inoculated with 1.0 ml. of a dilution selected to contain between 30 and 300 viable bacteria and two sets of thirty tubes of similar liquid medium were inoculated with 1.0 ml. of three effective dilutions differing by log one, each dilution being used to inoculate two sets of ten tubes. The sets of thirty were regarded as duplicates. The number of bacteria detected by both methods is recorded in Table X, which also shows the deviation of the duplicates.

TABLE X

NUMBER OF BACTERIA IN WANTER SAMPLES INDICATED BY DUPLICATE ANALYSIS BY THE SUCCESSIVE DILUTION METHOD AND PLATE COUNT PROCEDURES. THE DEVIATION OF DUPLICATES IS EXPRESSED IN PER CENT

	Successive Dilution Method				*Plate Count Procedure*			
Sample	*Number of bacteria*			*Per cent*	*Number of bacteria*			*Per cent*
No.	*A*	*B*	*Average*	*deviation*	*a*	*b*	*Average*	*deviation*
1701	239	310	274	25.9	263	231	247	12.9
1702	174	94	134	59.6	157	140	148	11.5
1703	197	115	156	52.5	113	120	116	6.0
1704	79	170	114	79.9	130	133	131	2.3
1705	275	334	304	19.4	263	228	245	14.3
1706	70	93	81	28.4	115	105	110	9.1
1707	101	231	166	78.3	194	163	178	17.4
1708	128	253	190	63.6	143	115	129	21.7
1709	381	116	248	106.7	225	241	233	6.8
1710	228	329	278	36.4	263	241	252	8.7

The successive dilution method indicated the presence of an average of 12.5 per cent more bacteria than the plate counts but the results

show that the reproducibility of the plate counts was five times as great as that of the dilution method counts. The plate counts deviated from the mean of the two duplicates by 2.3 to 21.7 per cent, the average for the ten samples being 11.1 per cent. The successive dilution method counts deviated from the mean of the two duplicates by 19.4 to 106.7 per cent, the average for the ten samples being 55.1 per cent. Halvorson and Ziegler (1933) present theoretical considerations which show that when three effective dilutions are used to inoculate ten tubes each, the results might deviate from the modal values by 50 to 130 per cent. They conclude that over a hundred tubes would have to be inoculated with three effective dilutions in order to have the reproducibility of the results approach that commonly obtained by plating procedures.

These observations seem to warrant the conclusion that the advantage of the slightly larger number of bacteria in marine materials indicated by the successive dilution method is more than offset by its poor reproducibility except when excessively large numbers of tubes are used. The relatively large amounts of time and material ordinarily required by the successive dilution method makes it impractical except for estimating the abundance of specific types of microorganisms with differential media.

CHOICE OF A SOLIDIFYING AGENT

Rendering sea water media sufficiently solid for plate counts and other cultural purposes presents special problems. If agar is used, the media commence to congeal at 42° C. at which temperature some of the thermosensitive bacteria from the sea are killed almost instantly (ZoBell and Conn, 1940). Nutrient gelatin can be prepared so that it does not congeal until its temperature has been reduced lower than the thermal death point of any known marine bacteria but the actively proteolytic bacteria which constitute a large portion of the marine flora liquefy the gelatin causing colonies to merge before many of the slower growing bacteria have time to form macroscopically visible colonies. Sea water media can be solidified with silica gel but plate counts thus obtained do not compare favorably with those obtained with media solidified with agar. Cognizant of these difficulties Bertel (1936) has recommended the use of the skeletal material of sponges which has certain applications but it is not suitable for general plate counts and other cultural purposes. Similarly bentonite, calcium sulphate, kieselguhr, cellulose and other solidifying agents have a strictly delimited usefulness. In our quest for a general utility medium for marine bacteria comparative tests have been made on different solidifying agents.

A few preliminary tests on the available materials with which we are familiar limited the choice of a solidifying agent for nutrient media to agar or gelatin although unexpectedly good results were obtained with silica gels. When prepared with freshwater by the method outlined by Hanks and Weintraub (1936) sufficiently firm silica gels contain around 3.5 per cent of sodium chloride which is formed when the sodium silicate is neutralized with hydrochloric acid. Since sea water contains nearly this much sodium chloride, it is not surprising to find that many marine bacteria will grow in properly enriched silica gel media without the necessity of dialyzing them to remove the sodium chloride as is commonly practiced when freshwater bacteria are to be grown on silica gels. Using a procedure similar to that described by Moore (1940) it has been possible to prepare and sterilize a solution of sodium silicate which produces a suitable gel which sets slowly when mixed with a second solution containing dilute hydrochloric acid and peptone or other nutrients. These two sterile solutions are mixed and poured immediately into a Petri dish inoculated with marine bacteria. The gel sets within a few minutes but not until there has been adequate time to permit the inoculum to be mixed with the sample.

About half as many colonies develop on nutrient silica gels prepared and inoculated in this manner as on plates of nutrient sea water agar (see Table XI). The media used in the experiments summar-

TABLE XI

Relative Numbers or Marine Bacteria which Formed Colonies on Nutrient Silica Gel Medium and Corresponding Nutrient Sea Water Agar and Nutrient Sea Water Gelatin after Different Period of Incubation at 22° C.

Period of incubation	*Nutrient silica gel medium*	*Nutrient sea water agar*	*Nutrient sea water gelatin*
4 days	13	38	42
7 days	22	71	63
10 days	30	89	68
14 days	37	97	—
18 days	46	100	—
24 days	54	95	—

ized in Table XI all contained 0.3 per cent Bacto-peptone, 0.2 per cent proteose peptone and 0.01 per cent ferric phosphate. The colonies which developed on the nutrient silica gel medium were smaller in size and exhibited less diversity of form than those which developed on nutrient sea water agar. The superiority of the sea

water agar is probably attributable to the specific salt requirements of the marine bacteria rather than being due to any preference of the bacteria for agar over silica gel because from the experiments summarized in Table III it will be recalled than only 56 to 61 per cent as many marine bacteria developed in a medium prepared with 3.0 per cent sodium chloride as in that prepared with sea water, agar being the solidifying agent in both cases. However, the attempts to improve the silica gel media by adding sea water to the solutions used to prepare the silica gel have failed to yield satisfactory results to date. A heavy precipitate forms when sodium silicate is added to sea water, and while the nutrient hydrochloric acid solution can be prepared with sea water, any advantages which accrue from the increased concentration of sea salts seem to be offset by the inimical effects of the salinity of the resultant medium. Though inferior to sea water agar for general plating procedures, silica gel media prepared by the method outlined above with appropriate nutrients have proved to be useful for isolating and studying the characteristics of certain marine microorganisms. Dialyzing plates of silica gel in sea water enriched with the proper mineral salts has given excellent media for the growth of marine nitrifiers, sulphur bacteria and diatoms.

The results summarized in Table XI show that marine bacteria develop somewhat faster in nutrient sea water gelatin than in a corresponding medium prepared with agar as the solidifying agent. A total of 24 samples of raw sea water and marine sediment have been analyzed in this way and after 4 days incubation there were 11.4 per cent more colonies on the gelatin plates than on the agar plates. However, after 7 days incubation when appreciably more colonies had appeared on both media, the agar excelled and the margin of its superiority increased progressively thereafter. Gelatin liquefaction by some colonies was evident in four days and after 7 days it had become so extensive that surrounding colonies were engulfed by expanding liquefied areas. The gelatin in some plates was quite completely liquefied after 10 days incubation at 22° C. and the colonies on only those plates which were inoculated with relatively small numbers of bacteria could be counted after 14 days.

Corroborating the observations of ZoBell and Conn (1940) it was found that the number of colonies on the nutrient agar continued to increase until the maximum was reached in about 18 days although there was little difference between the counts made on the fourteenth to the twenty-fourth day. The decreasing colony count observed after the eighteenth day is due to the obliteration of small colonies by rapidly growing ones.

Three or four per cent of the bacteria from the sea on the average

digest agar; a much larger proportion being found in plankton tows than in other marine materials. As a rule they do not liquefy agar as rapidly or as extensively as the proteolytic bacteria liquefy gelatin. Agar digesting bacteria usually form depressions in the nutrient agar, the diameter of the craters thus formed slowly increasing in diameter. Only infrequently is a plate of agar rendered completely uncountable by the activities of agar digesting bacteria. Angst (1929), Lundestad (1928), Waksman and Bavendamm (1931) and others have discussed the occurrence and activity of agar decomposing bacteria in the sea.

The fact that bacteria develop a little faster on nutrient gelatin than on nutrient agar during the first few days of incubation suggests that gelatin might supply some essential nutrient. According to Koser et al. (1938) gelatin is a good source of growth-promoting substances for bacteria. However, the addition of concentrations of gelatin ranging from 0.1 per cent to 1.0 per cent to nutrient agar failed to increase the plate counts or to improve results in any way.

From these experiments it is concluded that while agar is not a perfect solidifying agent for media for marine bacteria, it is better than anything else which is available. Vast differences were noted in the ease of dissolving in sea water and geling powers of different brands of agar as well as in the degree of clarity of the resulting medium. In all three respects Bacto-agar was superior. As little as 1.0 per cent of Bacto-agar is enough to render sea water medium solid enough for plate count purposes although it sets more firmly if 1.2 to 1.5 per cent is used. The concentration of agar from 1.0 to 2.0 per cent does not influence the number of colonies which develop.

ORGANIC NUTRIENTS

A survey of the literature reveals that scores of different concentrations and combinations of organic nutrients have been used for investigating the occurrence and distribution of bacteria in the sea. Peptone, beef extract, fish infusions, nutrose, gelatin, starch, dextrose and organic acids have been most extensively used constituents. The growth promoting properties of these constituents alone or in combinations including several dozen combinations specifically recommended for the multiplication of marine bacteria have been compared with our Medium 2216 consisting of 0.5 per cent Bacto-peptone, 0.01 per cent ferric phosphate and 1.5 per cent agar dissolved in sea water. Most of the media recommended by Russell (1893), Fischer (1894), Gran (1902), Drew (1911), Korinek (1926), Lipman (1926), Lloyd (1930), Levine and Schoenlein (1930), Bavendamm (1932),

Gee (1932), Bedford (1933), Benecke (1933), Reuszer (1933) and Waksman et al. (1933) for the growth of marine bacteria as well as various modifications have been tested. In the interests of the economy of space may it suffice to state that without exception all were found to be inferior to Medium 2216, there being only from 2 to 86 per cent as many bacteria growing on them as on Medium 2216 and, in general, fewer species.

One of the best media tried was one recommended by Reuszer (1933) and used by Waksman et al. (1933), Renn (1940) and others at the Woods Hole Oceanographic Institution. It consists of 0.1 per cent each of peptone and dextrose, 0.005 per cent K_2HPO_4 and 1.5 per cent agar dissolved in sea water. In a large number of comparisons it was found that only from 53 to 78 per cent as many colonies developed on this medium after different periods of incubations as on our formula 2216. Upon the addition of 0.01 per cent ferric phosphate to Reuszer's formula it gave plate counts which were an average of 86 per cent as high as those obtained on Medium 2216.

The effect of dextrose and the concentration of peptone on plate counts is illustrated by the data summarized in Table XII. The

TABLE XII

Comparative Plate Counts on Ferric Phosphate Sea Water Agar Prepared with Different Concentrations of Bacto-peptone without and with 0.1 per cent Dextrose

Medium	*Plate count without dextrose*	*Plate count with dextrose*
0.10 per cent peptone	72	86
0.25 per cent peptone	87	90
0.50 per cent peptone	100	102
1.0 per cent peptone	93	94
2.0 per cent peptone	96	91
Reuszer's medium (No Fe)	—	69

best results were obtained on media containing around 0.5 per cent Bacto-peptone. Dextrose is beneficial if the concentration of peptone is low but no advantages were noted for the use of dextrose in media containing as much as 0.5 per cent peptone. There is much more tendency for the colonies to be mucoid or to spread with subsequent merging on media containing more than 0.5 per cent peptone or on that enriched with glucose.

Some of the media used in the work of ZoBell et al. (1933, 1936, 1940) have been enriched with proteose-peptone, neopeptone, tryptone

or beef extract on the theory that these substances might favor the growth of certain fastidious organisms. While either alone or in combination with Bacto-peptone these substances have proved to be useful for the cultivation of pure cultures, our extensive experiments indicate the use of none of them for obtaining the maximum development of colonies (total numbers as well as kinds). In concentrations not exceeding 1.0 per cent they are not inimical except that beef extract imparts an undesirable brownish color to the medium.

Starch, glycerol, mannitol, citrates, lactates, succinates and malates have been recommended for the enrichment of media for the growth of marine bacteria by certain workers. The addition of from 0.1 to 1.0 per cent of the compounds to Medium 2216 has not improved it although studies on pure cultures to be reported elsewhere show that these compounds are utilized by certain bacteria and they are most useful constituents of differential media.

The work of Ostroff and Henry (1939) with pure cultures proves that marine bacteria are not fastidious in their carbon or nitrogen requirements. Some of their cultures could utilize as many as 14 of 21 different nitrogen compounds investigated and most of the bacteria grew luxuriantly on amino acids; glutamic acid, aspartic acid and asparagine being best in the order named. It was not within the scope of their work to try peptone which is a mixture of many amino acids and other nitrogen compounds. We find that neither glutamic acid, aspartic acid nor asparagine is as good as peptone for the cultivation of the general marine bacterial population. The enrichment of peptone media with 0.01 per cent of these amino acids either alone or in combination did not improve it for plate count purposes.

Snow and Fred (1926) found sodium caseinate agar to be superior to any other medium they tried for the cultivation of freshwater bacteria. Other workers have recommended the use of nutrose or sodium caseinate either with or without additional nutrients for the cultivation of marine or fresh water bacteria. The results which we obtained with the use of sodium caseinate and Bacto-nutritive caseinate are summarized in Table XIII. The average plate counts of four samples of sea water and three of marine sediments are given expressed as ratios on a basis of the plate count on Medium 2216 being 100. From the table it will be noted that only about half as many colonies appeared on the sodium caseinate media as on the peptone medium and those which did appear were much smaller and developed slower. The Bacto-nutritive caseinate was inferior to sodium caseinate in these tests. Many colonies on the caseinate medium might have escaped detection due to their small size and the opaque-

TABLE XIII

COMPARATIVE NUMBER OF MARINE BACTERIA WHICH FORMED COLONIES ON SEA WATER IRON AGAR ENRICHED WITH VARIOUS NUTRIENTS AFTER DIFFERENT PERIODS OF INCUBATION AT 22° C.

Nutrients	*Comparative plate count after 7 days*	*14 days*
0.2% sodium caseinate	19	46
0.5% sodium caseinate	32	53
1.0% sodium caseinate	37	54
0.2% sodium caseinate + 0.5% peptone	51	68
0.5% sodium caseinate + 0.5% peptone	56	71
1.0% sodium caseinate + 0.5% peptone	38	49
0.2% nutritive caseinate	15	23
0.5% nutritive caseinate	19	32
1.0% nutritive caseinate	20	38
0.5% peptone (Medium 2216)	86	100

ness of the media. The opaqueness of the media is also largely responsible for the fact that not as many colonies were found on the caseinate media enriched with peptone as on the peptone medium although it is not improbable that the mineral balance of the sea water was disrupted by the caseinate precipitating certain ions. The plate counts on the peptone medium were much more closely reproducible than on the caseinate media as indicated by the agreement of duplicates.

Considerable difficulty was encountered in trying to obtain reproducible results with various fish and seaweed infusions. In no case did any of four different fish and mollusk infusions or extracts either with or without peptone yield plate counts which were as large as those obtained without the infusions or extracts. Fish bouillon agar prepared with sea water according to the methods of Fischer (1894) was decidedly inferior to our standard peptone agar. Negative results were likewise obtained with the extracts prepared from two different red algae and three brown ones. Similarly after trying the extracts from marine organisms Lloyd (1930) concluded that they are not essential for the growth of marine bacteria in spite of the claims of many earlier workers.

The recent reviews of Koser and Saunders (1938) and Janke (1939) stress the importance of accessory growth factors, many of which are organic in nature, for the multiplication and metabolism of microorganisms. Harmsen and Verweel (1936) and others have recommended the enrichment of plating media with growth-promoting substances such as yeast extract or soil extract. The unpublished ob-

servations of Michener and others in the S.I.O. laboratory indicate that marine bacteria require certain accessory growth substances similar to those required by terrestrial and fresh water bacteria but apparently there is enough in sea water to initiate the growth of marine bacteria. Traces of nicotinic acid, thiamin chloride, pantothenic acid, indolacetic acid and yeast extract added to our ferric phosphate peptone Medium 2216 have not increased the plate counts although as will be reported elsewhere, some of these substances are beneficial for the growth of pure cultures in synthetic media.

SUMMARY

Most of the bacteria recovered from sea water as well as bottom sediments at places remote from possibilities of terrigenous contamination have specific salt requirements which are satisfied best by natural sea water. Neither synthetic sea water nor other isotonic salt solutions are satisfactory substitutes. Merely diluting sea water with fresh water materially reduces the number of marine bacteria which will grow in it. Conversely very few terrestrial or freshwater bacteria are able to grow in nutrient sea water media upon initial isolation.

There is no evidence that the addition of nitrate to nutrient sea water media as has been recommended by others is beneficial, and concentrations exceeding 0.1 per cent potassium nitrate are inhibitory.

There are indications for the addition of 0.01 per cent dibasic potassium phosphate to peptone sea water media although it normally contains sufficient phosphate to provide for the multiplication of marine bacteria.

The enrichment of nutrient sea water media with a trace of iron increases plate counts 18 to 76 per cent, 0.01 per cent ferric citrate or ferric phosphate being almost equally beneficial. The use of the latter is recommended because it provides for the phosphate requirements as well as the iron. This concentration of iron in sea water is toxic only when the medium is acid in reaction.

Marine bacteria appear to grow best in media having a pH of 7.5 to 7.8 although sea water *in situ* is usually somewhat more alkaline than this.

Successive dilution method counts using tubes of nutrient sea water broth demonstrated the presence of 12 per cent more bacteria than plate counts on comparable nutrient sea water agar but the latter yielded much more reproducible results and require much less medium and time.

A comparative study of the merits of different solidifying agents

recommends the use of 1.2 to 1.5 per cent Bacto-agar as the best for plating media. Gelatin is liquefied too rapidly by marine bacteria to be satisfactory and although silica gel prepared by a simplified method has certain advantages, it does not give results as good as agar for the demonstration of the heterotrophic bacterial population.

The use of various algae and fish infusions, carbohydrates, organic acids, nitrogen compounds and other organic nutrients in different concentrations and combinations have not proved to be as good as Bacto-peptone for the growth of marine bacteria as indicated by the number and kinds of bacteria on plates as well as the reproducibility of plate counts.

Although special media are required for certain groups of organisms and for special purposes, for maximum plate counts and for the cultivation of most aerobic heterotrophic marine bacteria Medium 2216 is recommended. It contains 0.5 per cent Bacto-peptone, 0.01 per cent ferric phosphate and 1.5 per cent Bacto-agar dissolved in aged sea water, the pH being around 7.6 after autoclave sterilization.

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ACKNOWLEDGEMENT

My chief collaborators in these investigations have been D. Q. Anderson, Catharine B. Feltham, H. D. Michener and S. C. Rittenberg whose contributions are gratefully acknowledged.

THE DETERMINATION OF DISSOLVED ORGANIC NITROGEN IN SEA WATER*

By

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The dissolved organic nitrogen occurring in the sea has so far received the least attention of all the nitrogen-containing fractions. As Krogh (*1*) has pointed out, it is present in relatively large amounts but is apparently unavailable for use by living organisms. The present investigation was carried out to test the practicability of a more extensive study of the distribution and significance of the dissolved organic N in the sea.

The method employed has been described by Krogh and Keys (*2*). A sample of 5 ml. of sea water is introduced into a silver combustion tube, which contains a pellet of pure KOH and through which a stream of purified oxygen-free hydrogen is passed. The extended tip of the tube dips into 1 ml. of dilute HBr solution. The water is first distilled off by gentle heating, after which the tube is heated along its entire length to about 500° C., which transforms the organic nitrogen into ammonia. Then 2 ml. of "nitrogen-free" water are introduced and the process repeated, since the salt residue tends to retain some ammonia. The ammonia driven over into the HBr solution is then determined according to Krogh's modification of Teorell's method (*3*). In a separate sample of sea water pre-formed ammonia must be determined according to Krogh, and this value subtracted from that obtained in the total nitrogen determination. A blank correction must also be applied, since it is impossible to procure water entirely free of dissolved organic N. It occasionally happens that, for some reason, the ammonia is incompletely driven off. To guard against this source of error a blank run on 2 ml. of the "nitrogen-free" water is made after the analysis of each sample.

We found that it is possible with this procedure to carry out a series of four analyses and blanks without opening the combustion tube.

The method requires practice before satisfactory results are achieved. In Table I we give the analytical data from two sets of determinations, in order to illustrate the accuracy that can be

* Contribution No. 270 from the Woods Hole Oceanographic Institution.

TABLE I

Comparison of Successive Determinations on Two Different Samples of Sea Water. One Microgram of N Corresponds to 0.56 ml. of Naphthyl Red

Sample	Titration: ml. of naphthyl red			Dissolved organic
	Blank	5 ml. sea water	Diff.	$N+NH_3$ in γ per l.
1	2.96	2.27	0.69	246
1	2.95	2.39	0.56	200
1	2.95	2.26	0.69	246
1	2.97	2.35	0.62	222
			Mean	229
2	3.80	3.28	0.52	186
2	3.84	3.28	0.56	200
2	3.79	3.27	0.52	186
2	3.81	3.30	0.51	182
			Mean	189

expected. The first is a set of average accuracy that can be obtained without great difficulty; the accuracy of the second series is obtained only after long practice, and is even then not always achieved.

A further test of the accuracy of the method was made by analyzing 6 samples of sea water 16 times each, in sets of 4 analyses at a time. All these samples had been stored in the laboratory, at room temperature and in the light, for over one year. Aged sea water was chosen, since it could be assumed that no further changes in the content of dissolved organic N would occur during the time of the analysis (1 day to 1 week). It could therefore be expected that differences in the results were due to analytical errors only. We have then calculated the mean value of the 16 determinations of each sample and the percentage deviation of each set of 4 analyses from this mean. Table II shows that the error of a set of analyses will in

TABLE II

Average Deviations in per cent. in Six Different Samples of Sea Water. Each Series Contains Four Analyses. Average Deviations from the Mean of Sixteen Determinations

Station	*3354*	*3515*	*3245*	*3245*	*3245*	*Misc.*
Depth	*0m*	*0m*	*0m*	*800m*	*1200m*	*>1000m*
Series 1	+3	− 5	−5	−11	+5	−8
Series 2	−3	+12	±0	+ 6	−4	+4
Series 3	−3	− 9	+2	− 4	±0	−2
Series 4	+3	+ 3	+3	+ 8	−1	+6

general be well below 10%. The accuracy of the method should be enough to decide whether or not the distribution of dissolved organic N is as uniform as Krogh assumes. Table III shows the absolute values found in these and in two other samples of aged sea water. These are of some interest, since they can be compared with Krogh's fresh samples. The average of our four surface samples is 147 γ per liter; that of the 4 deep samples 214 γ. This latter figure is close to Krogh's value of 244 γ for a whole deep water column, possibly within experimental error. The values for surface water are, however, decidedly lower. Whether this is due to a stratification of the dissolved organic N in the sea, or to disappearance of nitrogenous substances during storage, must be decided by later observations.

TABLE III

Dissolved Organic N in Aged Sea Water Samples
Micrograms per Liter

Station	*Depth m*	*Age of sample*	*Organic* $N+NH_3$	NH_3	*Dissolved organic N*
3354	0	16 mo.	158	0	158
3515	0	15 mo.	111	6	105
1474	0	7½ yrs.	173	45	128
3245	0	18 mo.	266	70	196
3245	800	18 mo.	238	16	222
3245	1200	18 mo.	249	10	239
1474	3000	7½ yrs.	216	8	208
Misc.	>1000	22 mo.	185	0	185

The method can hardly be used on ship board. It was therefore necessary to ascertain the conditions under which samples can be satisfactorily preserved for analysis after return to the laboratory. Tests were carried out on two samples of harbor water, which were analyzed immediately after sampling and again after storage for various periods under conditions indicated in Table IV. In the case of filtered samples stored in the ice box, or kept either in the dark or the light at room temperature, it seems likely that the differences between the single sets of analyses are due to analytical errors rather than to actual changes. The same applies probably to the unfiltered sample kept in the light. If the content in particulate matter is high, however, and if such a sample is stored in the dark, a considerable, but slowly diminishing, amount of dissolved organic N accumulates. This is due to the fact that under these conditions the organisms die quickly and a large part of their body substance

TABLE IV

DISSOLVED ORGANIC N IN SAMPLES OF SEA WATER STORED UNDER DIFFERENT CONDITIONS. NOT FILTERED. IN LIGHT AT ROOM TEMPERATURE

Conditions	Storage time, days	Micrograms per liter		
		Organic $N+NH_3$	NH_3	Dissolved organic N
*Filtered**	0	147	23	124
Dark	7	177	16	161
In refrigerator	12	158	20	138
	22	146	17	129
	28	170	16	154
Not filtered	0	147	23	124
Light	14	168	8	160
Room temperature	23	151	10	141
	28	175	4	171
*Filtered**	0	189	80	109
Dark	6	170	37	133
Room temperature				
*Filtered**	0	189	80	109
Light	6	129	9	120
Room temperature				
*Filtered**	0	189	80	109
Dark	7	152	22	130
In refrigerator				
Diatoms added	0	189	80	109
(318 γ/liter)	4	507	138	369
Dark	9	486	209	277
Room temperature	14	545	265	280

* With No. 4 sintered-glass filter.

goes into solution. Later, it is transformed into ammonia by bacterial activity.

For future investigations therefore, we recommend that samples of about 250 ml. be immediately filtered through No. 4 sintered glass and stored at once on ice in glass-stoppered bottles. Analysis should be carried out as soon as possible. Under these conditions differences of the order of 50 γ per liter are significant.

We acknowledge the help of Dr. J. W. Zabor in carrying out some of the ammonia determinations.

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INTERNAL WAVES IN THE GULF OF CALIFORNIA*

By

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Between February 13 and March 19, 1939 the "E. W. Scripps," research vessel of the Scripps Institution of Oceanography occupied fifty-three oceanographic stations in the Gulf of California. On the basis of observations of temperature and salinity which were taken at every station at a series of depths a chart of the dynamic topography of the surface relative to the 1500-decibar surface was derived, but this chart does not indicate any regular exchange of water between the Gulf and the adjacent part of the Pacific. It shows a sequence of separate highs and lows which are associated with alternating downward and upward displacements of the isopycnic surfaces and suggests the existence of internal waves with amplitudes as one-tenth the depth of the Gulf.

Let ρ be the density, depending upon the temperature and salinity of the water. The vertical displacements, as represented by σ_t curves ($\sigma_t = 10^3(\rho - 1)$ are clearly seen in a longtitudinal section (Figure 7) based on data from stations along the center line of the Gulf, as shown on the small map to the left in the figures. It should be observed that the vertical scale is greatly exaggerated relative to the horizontal, the ratio between vertical and horizontal scales being equal to 1/800. The profiles of a series of isobaric surfaces relative to the 1500-decibar surface are shown to the right in the figure.

These observations have been discussed by H. U. Sverdrup (1940; in press) who concludes that the wavy character of the isobaric surfaces might be due to the presence of a standing internal wave, the period of which would lie between 6.3 days and 7.65 days, probably closer to the former value. This explanation is consistent with boundary conditions. The wave would be of first (fundamental)

*Contributions from the Scripps Institution of Oceanography, New Series, Number 123.

Assistance in the preparation of this paper was furnished by the personnel of the Works Progress Administration, Office Project No. 65-1-07-2317.

order with respect to the vertical axis, the vertical displacement vanishing at surface and bottom only, and three nodes inside the Gulf make it of fourth order with respect to the horizontal axis. A node at the mouth of the Gulf indicates that one has to deal with a free oscillation. The wave length of 1000 kilometers near the mouth of the Gulf indicates that the two progressive waves which combine to the standing wave there have velocities of propagation of 175 cm/sec which decrease towards the closed end of the Gulf. For further description of these waves and internal waves generally refer-

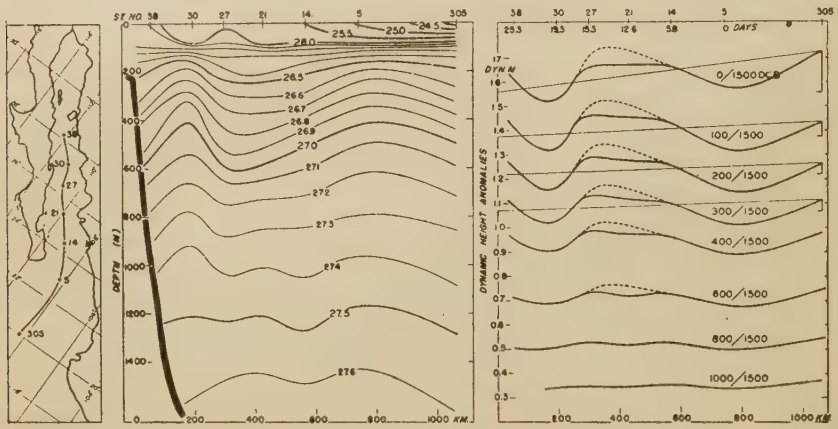

Figure 7. Vertical distribution of density (σ_t) and profiles of isobaric surfaces referred to the 1500-decibar surface along a section in the Gulf of California, as shown on the map at the left in the figure. (After H. U. Sverdrup.)

ence should be made also to Defant (1929), Sverdrup, Johnson and Fleming (in press), and W. H. Munk (in press).

Internal waves are accompanied by large horizontal velocity components at the bottom. It will be shown later (Figure 9) that the horizontal velocity at the bottom of the Gulf attains a maximum value of 20 cm/sec which is sufficiently large to exert a pronounced influence upon the processes of sedimentation. Within a standing wave the horizontal velocity component reaches a maximum near the location of nodes and one may expect that only large-sized particles settle in their vicinity. On the other hand, anti-nodes are characterized by very small horizontal currents which would permit small particles to be deposited at the bottom.

Such an influence of internal waves upon the distribution of sediments was pointed out by R. R. Revelle (1939) who found the aver-

age median diameters of bottom samples in the Gulf to vary in a regular manner along a north-south direction, corresponding to an internal wave with three nodes. This evidence is not conclusive, but it suggests that the particular wave-form of fourth order with respect to the horizontal axis does not constitute an isolated phenomenon but appears to be of common occurrence. The evidence would be greatly strengthened if it could be shown by means of theoretical considerations that the observed oscillation represents one of the possible natural oscillations in the Gulf.

Such a wave might be caused by an outside periodic disturbance the period of which would have to be closely related to one of the free periods of oscillation in the Gulf. The lunar-fortnightly tide has a period of 13.6 days and a theoretical amplitude which is one sixth the amplitude of the principle semi-diurnal lunar tide. If the length of one of the natural periods of the Gulf would correspond closely to 13.6 days an oscillation with this period would be dominant, but if a natural period close to 13.6 days does not exist, the lunar-fortnightly tide may cause an oscillation of approximately half this length, 6.8 days, corresponding to the observed period of oscillation of the Gulf.

Hence, it will be necessary to investigate theoretically the question whether the picture which has been derived from observations is in agreement with theoretical considerations and whether lunar forces may be responsible for the formation and maintenance of an internal wave of the observed type.

A body, defined by its distribution of mass and its dimensions, has a definite set of natural frequencies. These frequencies can be easily calculated in the case of uniform bodies of simple geometrical shape but the corresponding calculation becomes very complicated when applied to the waters of the Gulf of California within which the distribution of density is a function of depth (Figure 9) and the boundaries of which are determined by the topographic features of the Gulf (Figure 8). J. E. Fjeldstad (1933) has developed the theory of progressive internal waves in a channel of constant depth, provided that the density distribution does not change in a horizontal direction. W. H. Munk (in press) has treated the cases of progressive or standing waves, assuming constant depth and appreciable horizontal density gradient and of standing waves, assuming variable depth but negligible horizontal density gradient. For rigorous derivations reference is made to these papers, and here it will be attempted only to outline the latter theory, to explain its application, and to discuss the results pertaining to the Gulf.

Previous investigations of wave phenomena (Defant, 1929) have

shown that in a long narrow channel the earth's rotation will tend to produce oscillations in a plane perpendicular to the length of the channel, but its effect upon the chief wave motion along the channel will be small and can in first approximation be neglected. For that reason the problem reduces to a two-dimensional analysis.

We shall assume the x-axis along the direction of propagation of the wave, the z-axis perpendicular to the surface, where $z = 0$ represents the undisturbed surface, $z = H$ the bottom. (Figure 8.) Let u and w represent the components of the velocity of the water along the x and z axes respectively, ρ the density, p, the pressure, and η the vertical displacement. Let $\rho_0(z)$ denote the density in equilibrium condition and define ρ_1 by

$$\rho = \rho_0(z) + \rho_1(x, z, t)$$

Let

$$p_1 = g \int_0^z \rho_1(x, z, t)$$

Neglecting second-order terms, the fundamental equations of hydrodynamics take the form

$$\frac{\partial u}{\partial t} + \frac{1}{\rho_0}\frac{\partial p_1}{\partial x} = 0 \tag{1a}$$

$$\frac{\partial w}{\partial t} + g\frac{\rho_1}{\rho_0} + \frac{1}{\rho_0}\frac{\partial p_1}{\partial z} = 0 \tag{1b}$$

$$\frac{\partial u}{\partial x} + \frac{\partial w}{\partial z} = 0 \tag{1c}$$

$$\frac{\partial \rho_1}{\partial t} + w\frac{\partial \rho_0}{\partial z} = 0 \tag{1d}$$

To investigate a progressive or standing wave with period $T = \frac{2\pi}{\sigma}$, we assume:

Progressive wave	*Standing wave*
$u\,(xzt) = U\,(xz)\sin(kx - \sigma t)$,	$u\,(xzt) = U\,(xz)\cos(\sigma t)$
$w\,(xzt) = W\,(xz)\cos(kx - \sigma t)$,	$w\,(xzt) = W\,(xz)\cos(\sigma t)$
$p_1(xzt) = P_1\,(xz)\sin(-kx + \sigma t)$,	$p_1\,(xzt) = P_1\,(xz)\sin(\sigma t)$
$\rho_1\,(xzt) = R_1\,(xz)\sin(-kx + \sigma t)$,	$\rho_1\,(xzt) = R_1\,(xz)\sin(\sigma t)$

(2)

Substitution of either set of assumptions into equation (1) eliminates the function of time, and the remaining four equations in four unknowns can be solved for $W(x\,z)$:

$$\frac{\partial^2 W(xz)}{\partial z^2} - g\frac{\varphi(z)}{\sigma^2}\frac{\partial^2 W(xz)}{\partial x^2} = 0 \tag{3}$$

where

$$\varphi = -\frac{1}{\rho_0}\frac{\partial \rho_0}{\partial z} \tag{3b}$$

The substitution $W(x\,z) = W(z)\sin(k\,x)$ reduces the partial differential equation (3) to the total differential equation:

$$\frac{d^2 W(z)}{dz^2} + \left(\frac{1}{c}\right)^2 g\,\varphi\, W(z) = 0 \tag{4}$$

where the wave length $\lambda = \frac{2\pi}{k}$ and the velocity of propagation, $c = \frac{\lambda}{T}$.

This is the differential equation derived by Fjeldstad for the case of constant depth. It can be shown, however (Munk, in press), that this substitution $W(x\,z) = f(z)\cdot F(x)$ is inconsistent with the general problem and consistent only for a basin of constant depth, wherefore, assumptions (2) leading to equation (3) must be used when treating the case of variable depth.

Assumptions (2) referring both to standing and progressive waves, satisfy the basic laws of hydrodynamics and lead to differential equation (3). This is true because the standing wave may be considered a superposition of two progressive waves of equal amplitude but opposite direction, and both progressive waves are proper solutions, hence their sum, the standing wave, must also be a solution. On the other hand, the two wave-forms behave in a very different manner with regard to boundary conditions and it can be shown (Munk, in press) that the solution referring to progressive waves cannot be applied to a basin of variable depth. This indicates that a standing wave can exist in a basin in which neither of the two progressive waves can exist, which may be considered as making up the standing wave.

In order to obtain values for the periods of natural frequency, σ must be computed from the differential equation (3), subject to boundary conditions:

$$W = 0 \text{ at } x = 0 \tag{5}$$

since a node is located at the mouth of the Gulf (Figure 7), and

$$W = 0 \text{ at } z = 0 \tag{6}$$

since the vertical motion caused by an internal wave at the surface is always small enough to be neglected. Finally, the kinematic boundary condition at the bottom, namely, that all flow must take place *along* the boundary, is expressed by

$$W = mU \text{ along the bottom, where } m \text{ denotes the slope.} \tag{7}$$

Let W be expanded in a Fourier series:

$$W(xz) = \sum_{i=1}^{n} W_i(z) \sin (k_i x) \dot{C}_i(o) \tag{8}$$

where $k_i = i\pi/2L$, L, the length of the basin, and $C_i(o)$ a constant so chosen that $\dot{W}_i(o) = 1 \left(\dot{W}_i(z) = \frac{dW_i}{dz}\right)$

With these substitutions we obtain for equation (3)

$$\frac{d^2W_i(z)}{dz^2} + \frac{g\,\varphi(z)k_i^2}{\sigma^2} W_i(z) = 0 \quad (i = 1, 2, \ldots n) \tag{9}$$

Boundary condition (5) is already satisfied. Boundary condition (6) gives

$$W_i(o) = 0 \tag{10}$$

Boundary condition (7), with the aid of the equation of continuity, yields

$$\sum_{i=1}^{n} \left[W_i(z) \sin (k_i x) + \dot{W}_i(z) \frac{m(x) \cos (k_i x)}{k_i} \right] \dot{C}_i(o) = 0 \tag{11}$$

In the Fourier summation (8), n must be at least equal to two in order to satisfy all boundary conditions of a basin of variable depth and must be larger than two to give a good approximation. By making n sufficiently large we can approximate as closely as we wish any velocity distribution corresponding to an observed wave.

We note that equation (9) represents n equations of the same type as equation (4), which Fjeldstad has developed for the case of constant depth. Each of these equations describes on internal wave of wave length $\lambda_i = \frac{4L}{i}$ and can be integrated numerically according to the method used by Fjeldstad (1933). In this manner we obtain values for $W_i(z)$ and $\dot{W}_i(z)$ at all depths. The internal wave can then be considered as the result of n internal waves of equal period but different wave lengths, superimposed upon one another.

In order to apply boundary condition (11) we shall divide the length L of the basin into r equidistant intervals with values of x ranging from $x_l = 0$ to $x_r = L$ (Figure 8). By means of an accurate topographic section of the basin one can find for all values of x_j $(j = 1, 2, 3 \ldots r)$ the corresponding slope m_j and depth z_j. Let us then *assume* a definite value for the parameter σ and obtain

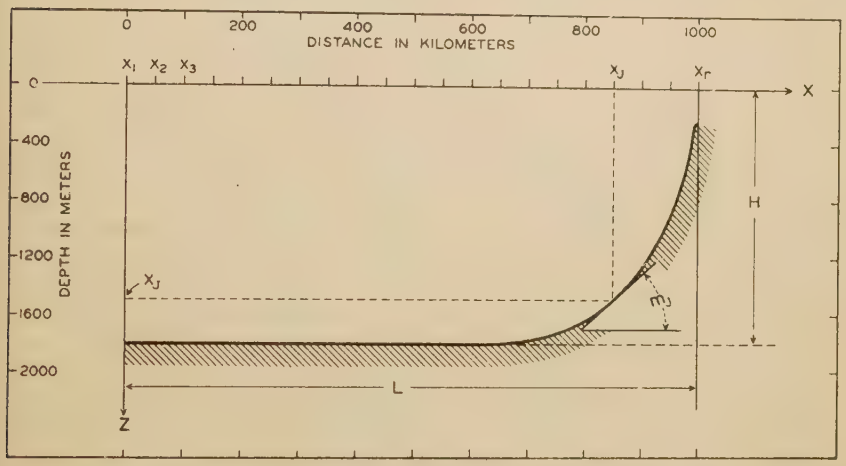

Figure 8. Schematic topography of the Gulf of California and notation used in numerical integration.

$W_i(z)$ and $\dot{W}_i(z)$. for all values of z_j by means of numerical integrations. If we define

$$K_{ji} = W_i(z_j) \sin(k_i x_j) + \dot{W}_i(z_j) \frac{m(x_j) \cos(k_i x_j)}{k_i} \tag{12}$$

boundary condition (11) becomes simply

$$\sum_{j=1}^{r} \sum_{i=1}^{n} K_{ji} \dot{C}_i(o) = 0 \tag{13}$$

Munk (in press) has devised a method to test how well boundary condition (13) is satisfied. It calls for the evaluation of a determinant D which will vanish for every value of σ which corresponds to a natural frequency. This, however, is true only for an infinite value of n, for otherwise a Fourier series will give an approximation only to the actual velocity distribution, and D will not vanish completely but will reach a minimum.

For $n = 3$,

$$D = \begin{vmatrix} \sum_{j=1}^{r} K_{j1}K_{j1} & \sum_{j=1}^{r} K_{j1}K_{j2} & \sum_{j=1}^{r} K_{j1}K_{j3} \\ \sum_{j=1}^{r} K_{j1}K_{j2} & \sum_{j=1}^{r} K_{j2}K_{j2} & \sum_{j=1}^{r} K_{j2}K_{j3} \\ \sum_{j=1}^{r} K_{j1}K_{j3} & \sum_{j=1}^{r} K_{j2}K_{j3} & \sum_{j=1}^{r} K_{j3}K_{j3} \end{vmatrix} \tag{14}$$

D can now be calculated for various values of σ and the natural frequencies can be determined because the value of D must be at a minimum when σ corresponds to a natural frequency.

The bottom topography of the Gulf of California is quite complicated but observations at the deepest stations indicate that to a considerable distance from the mouth continuous communication between the various depressions is present above 2000 meters. It seemed therefore justifiable to choose an "effective" depth which remains close to 1800 meters throughout the larger portion of the Gulf, but decreases regularly in the inner part.

At about 29° latitude the channel becomes very shallow and two islands, San Esteban and San Lorenzo, rise above the surface. Waves will be reflected before they reach this point or lose their energy by friction and interference. Hence we may consider this the inner end of the Gulf, and may neglect the portion north of the islands, although the Gulf deepens and widens again, and extends 350 kilometers further inland.

It is generally known that the "effective" length of a bay with regard to a wave phenomenon is not identical with its actual length, but depends upon its width at the open end. A correction formula has been derived which, in this case, gives an "effective" length of 1000 kilometers, roughly 20 per cent longer than the actual length.

Fjeldstad's theory for basins of constant depth was applied to the outer portion of the Gulf of California. Figure 9 illustrates the results obtained by this theory. They compare favorably with those obtained from observations.

	c	H
Theory	167 cm/sec	675 m
Observations	175 cm/sec	500–600 m

where c represents the velocity of propagation, H the depth of maximum amplitude of oscillation.

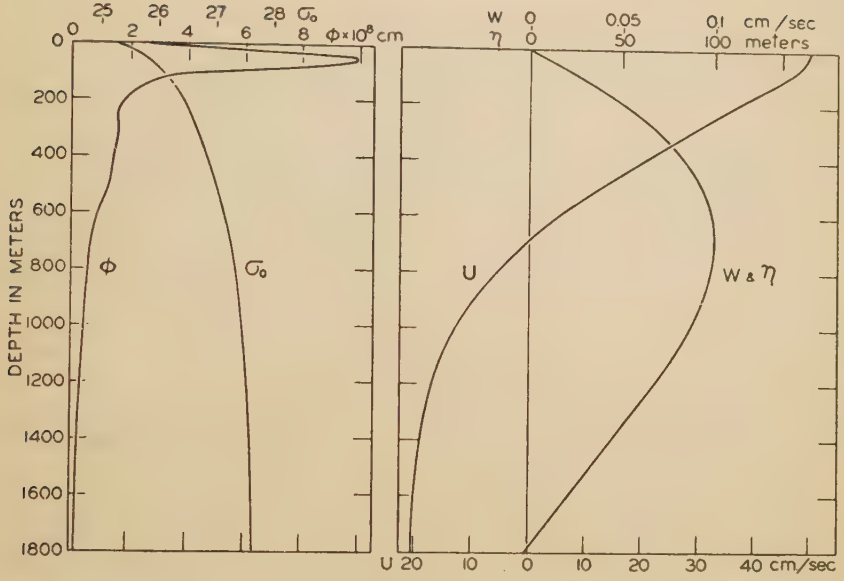

Figure 9. *Left:* Average vertical distribution of density and density gradient along the center line of the Gulf of California.

Right: Horizontal and vertical velocity components and vertical displacement as functions of depth in the outer portion of the Gulf of California.

The calculation of D was carried out for three values of i only. For investigating an approximately weekly period values of $i = 4$, 5, and 7, corresponding to wave-lengths near the mouth of 1000 km, 800 km, and 570 km were chosen, whereas in the case of the fortnightly period values of $i = 3$, 4, and 5 were chosen corresponding to wave-lengths of 1333 km, 1000 km, and 800 km. These particular components were most satisfactory because they gave a low value for D, as illustrated in the following tables:

	Values of i	*5-6-7*	*3-4-5*	*4-5-6*	*4-5-7*
T = 6.4 days					
	$D \times 10^{-27}$	130	123	63	25
	Values of i	*2-3-4*	*2-3-5*	*2-4-5*	*3-4-5*
T = 14 days					
	$D \times 10^{-27}$	26	54	6.4	4.0

A further examination (Munk, in press) shows that in both cases a wave dominates which near the mouth has a wave length of 1000

km, decreasing toward the closed end. The nodal regions of both waves must approximately coincide and the distribution of sediments may be consistent with the presence of either wave.

In a vertical direction the weekly wave is primarily of the first order, whereas the fortnightly wave is of the second order. The observed distribution of density indicates that a first-order wave dominates but the presence of a second-order wave is not excluded and

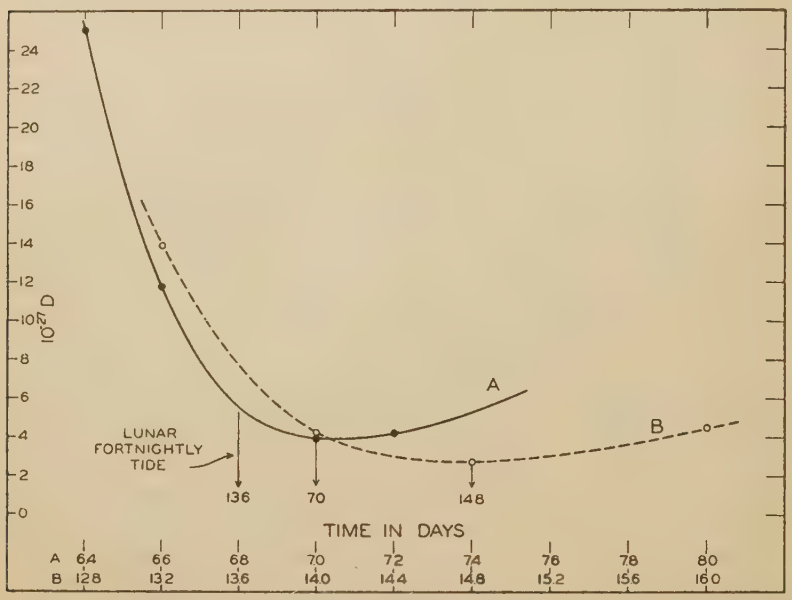

Figure 10. Results obtained by numerical integration indicating two periods in the Gulf of California of natural frequencies of 7 days and 14.6 days, respectively, and their relations to the lunar fortnightly tidal period.

may account for the fact that the observations appear to give a smaller value of H, the depth of maximum vertical oscillation.

The main results of the numerical integrations are presented in Figure 10 according to which minima correspond to periods of 7 days and 14.8 days. The fact that one minimum falls at a period of 7 days is in good agreement with the conclusions based on observations, particularly because the computed oscillation is, as already explained, of a character similar to the observed one. Twice this period of seven days comes closer to the lunar-fortnightly period of 13.6 days than the minimum obtained near 14.8 days, indicating that resonance is more likely to occur in the case of the seven-day period than in

the case of the 14.8 days, and that the seven-day period, therefore, may dominate. Thus, the theoretical examination fully confirms Sverdrup's interpretation of the observations from the Gulf of California.

The writer wishes to acknowledge the help of Dr. H. U. Sverdrup, who suggested this investigation and without whose advice and encouragement it could not have been completed. The writer is also indebted to Dr. H. Bateman for his help in finding a solution to the general differential equation, and to Dr. L. Lek and Mr. F. Brunner for having carried out many of the tedious calculations.

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THE PARACHUTE NET

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The novel idea of fishing a large net without a towing line was adapted from the method used by Professor Maurice Ewing of Lehigh University in his work on the sub-sedimentary rocks beneath the sea. The method consists essentially in dragging the apparatus to the bottom by a weight against the upward pull of a float and then releasing the weight. His successful demonstration off Bermuda in 1939 with twelve pieces of apparatus (three oscillographs, bomb-shooters and marking buoys) put overboard at half-mile intervals on a line three and a half miles long from a sea anchor buoy, showed that the independent method was practicable and could be applied to a number of oceanographic problems.

I wish to convey my acknowledgements and thanks to Professor Ewing, Dr. Woollard of Princeton and Mr. A. Vine of Lehigh, all of whom contributed to the solution of problems concerning flotation and release, whose devices I have appropriated; also to Mr. Chesterfield Burgess of the Biological Station staff for assistance in the construction of the net.

Bermuda is peculiarly fitted for the study of certain types of oceanic problem. The proximity of deep water to a well-equipped shore laboratory brings the possibility of physiological work on living deep-sea organisms nearer realization than elsewhere and has already allowed the certain identification of several deep sea crustacean larvae from successive moults of the living specimens. The difficulties of collecting material have been mainly concerned with the gear used—the unwieldy form of the large net which requires a number of men to handle it and a powerful winch with heavy wire to fish it at any depth, the time taken in hauling, especially damaging to the catch in the upper warmer water, and the shaking together of the animals in the ordinary open townet bucket in the wake of the net, if closed, or by turbulence in the bucket itself, if open. If the towed net touches the bottom it is usually lost with part of the towing wire so that great care is usually taken in towing large nets to fish them well off the bottom and considerable weights have to be used to take the net down to the required depth.

The parachute net evolved in three stages. In 1936 I was experimenting with a plankton trap-net for nocturnal migrants in shallow water. The net used was a fine silk ½-metre townet of the usual pattern with a weighted ring to hold the mouth downwards on the bottom. An air-filled float supported the bucket and a buoy was attached to mark the position. The bucket was a 1-gallon can made for carrying kerosene with a screw cap at the top. Through the bottom was soldered a tube extending through the can to near the top, having at its other end, outside the can, a funnel to which the cod-end of the net was attached. A small hole was punched in the cap to let out the trapped air. This apparatus was lowered to the bottom in Ferry Reach off the Station and it was hoped that the comparison of nightly catches taken for several months would reveal a lunar periodicity in organisms other than *Anchistioides, Penaeopsis,* and *Periclimenes* and perhaps provide a clue to the daylight hiding-places of the first-named. The trap-bucket successfully retained Copepods and other animals but the rapid fouling of the net and rotting of the silk brought the experiments to an end.

The success of Professor Ewing's independent method suggested the use of a net carried to the bottom and fishing on its upward path but the idea was soon abandoned since it obviously suffered from some of the defects of the towed net, i.e., slow ascent, damage to specimens and necessarily small size owing to the weight and cumbrous nature of a large ring and net. It was realized, however, that if the net were inverted and made to fish on its downward journey it would return rapidly to the surface provided that some means could be found of throwing off the ring. It was but a step to the conception of using the mouth of the net itself as a self-opening parachute and using the trap-bucket for holding the catch.

Preliminary experiments with a small net indicated that the mouth could be made to open. A large net was designed and tested in shallow water horizontally before the full vertical trial was made in 1300 fathoms. When this test was successful the net and bucket were altered in detail and a second test took place. The model used is nine feet three inches across the mouth and almost thirty-six feet in length from the cod-end to the ring of the bridles, yet it is paid out over the side for shooting and pulled in again like rope. It comes to the surface rapidly—the speed of ascent is estimated at more than six knots—and it fishes until the weight strikes the bottom. The catches have been remarkable for their perfect condition. The independent parachute net thus appears to solve some, at least, of the difficulties of deep sea collection.

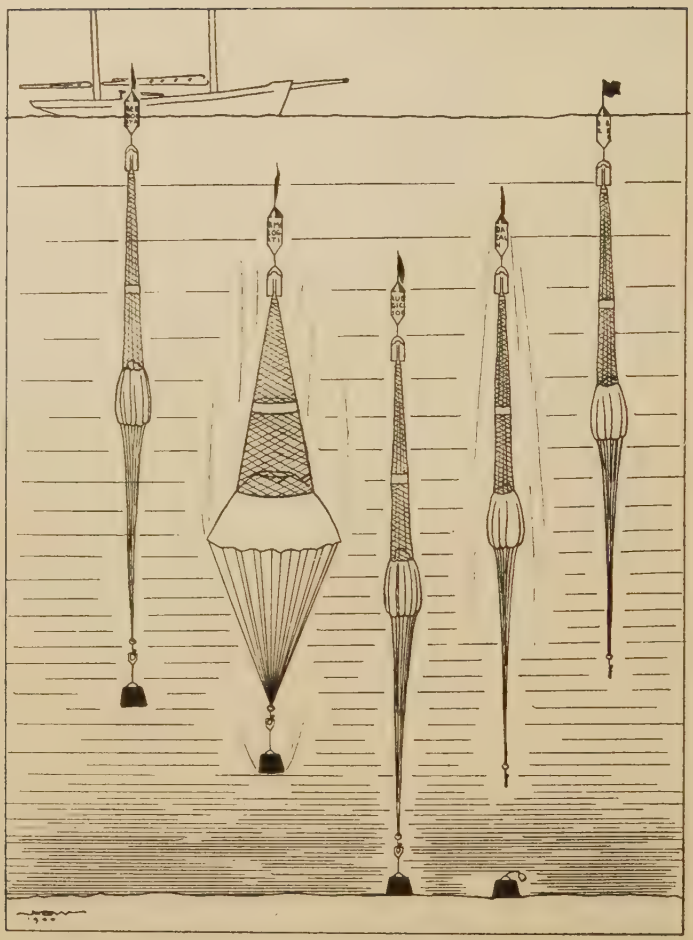

Figure 11. The parachute net.

In Figure 11 the stages of shooting, descent and ascent are shown. The weights used in the second test were two square concrete blocks moulded for Professor Ewing's experiments and weighing 68 and 65 lbs. in air. They were tied together and put over the side one after the other. A 'salt release'—a bag containing a block of salt held in a loop of galvanized wire—was then attached. I used a triple mosquito net bag and a 605 gr. block of salt. Then came the ring of the 16 bridles, 14 ft. 6 in. long, with a slipping rope passed through

it so that the weights could be lowered while the rest of the apparatus was put into the water. In the first test there were 19 bridles of light rope which I tried subsequently to reduce to ten. With this number the net opened twice in three attempts when shot from the launch so that sixteen appeared to be nearer the minimum number. The rope was replaced by heavy fishing line (72-strand) knotted outside the eyelets in the margin of the net at intervals of 21 ins. The net itself was built up from an old 1-metre mosquito netting townet with a 9½ inch canvas heading. The parachute mouth was sewn locally after being cut and pinned into shape. It was made of unbleached calico, known as 'Indian Head,' 36 ins. wide, trimmed into the curved form necessary to make a truncated cone 28 ft. 4 in. round the larger end, 15 ft. 4 in. round the smaller, and 4 ft. 10 in. deep. The smaller end was originally joined to the 1-metre net canvas with a 3 ft. 6 in. width of fish netting of large mesh (3½ inch pulled out diagonally), but this section was replaced by mosquito netting 6 ft. 8 in. deep before the second test.

When the net as described was towed from the launch it refused to open fully and a secondary cone was sewn into the back of the mouth leaving a circular hole 2 ft. 1 in. in diameter opening into the net. Still the mouth remained obstinately half-closed. It was then observed that water was filtering through the material as fast as the net could be towed, an unsuspected cause of failure which suggested as a parallel the disastrous results attendant upon aerial parachutes made of lace. That the analogy was true was confirmed by applying a coat of paint to the parachute mouth which then opened to its full extent and gave no further trouble. The secondary cone was left in place to act as a valve when the net touched the bottom. It was not painted. Both sections of mosquito netting were strengthened with four longitudinal lines (fishing line as used for the bridles) tacked to the netting in places and sewn to the parachute mouth, to the canvas heading of the 1 m. net and the cod-end of the latter. Here the four free ends were used for attachment to eyelets soldered to the bottom of the bucket and took the strain of the weights off the cod-end, which was slipped over and bound to the funnel of the trap. The bucket was a 2-gallon galvanized kerosene can with a plate soldered over the place from which the pouring spout had been removed. The funnel led into a straight pipe 3.3 cm. in diameter passing up the centre of the can and ending just under the level of the upper rim. A small hole was punched in the centre of the screw cap and five other holes were made over the top of the can, but the latter were stopped up for the second test and replaced by five ¼ in. holes close together on the side of the

can just under the rim covered with fine-mesh galvanized wire soldered into position. This change was made to avoid the churning action of jets of water squirting into the can on the upward journey. The centre hole was left to deal with trapped air and as the jet from it squirts into the pipe it cannot do any damage to the catch. The bucket was bound with wire, the original frail handle removed and a new one made of wire used for attachment to the float.

The float was a spindle-shaped galvanized tank with a capacity of 15½ gallons, made locally for the Ewing experiments. The flotation agent was gasoline. A small screw valve at the lower end of the tank allowed the pressure and temperature contraction of the gasoline to be directly equalized by sea water. I found that one gallon of gasoline was capable of lifting about four pounds (weighed in air) so that the full tank should be able to lift more than sixty pounds. The weights (in air) were: Gas tank, 15¼ lbs.; bucket, 4¼ lbs.; net and bridles, 16 lbs.; a total of 35½ lbs. The full tank, therefore, gave an ample margin of lift yet there was sufficient weight to make it float upright at the surface with its red-painted cone top fully exposed and the red flag that was lashed to its side visible at a considerable distance even under the somewhat severe conditions of wind and sea experienced during the second test.

The first test was carried out on September 5, 1940 from the research vessel 'Culver' operated by the Bermuda Oceanographic Committee of the Royal Society of London. A lowering on wire until the red cap of the float disappeared from sight gave a metre-wheel reading of 13 m., checked by Kelvin tube fastened against the float. The net was shot at 9.50 a.m., with two weights, totalling 134 lbs., and a salt release of 526 gr. calculated to dissolve in about 2¾ hours. The net opened perfectly almost directly it left the surface, swelling out into the form of a gigantic jellyfish and dwindling rapidly in the clear water. The red top disappeared in 26 seconds which gave an approximate time of 79 minutes for the 1300 fathom descent. The flag and float were seen to come to the surface at 12.10 p.m. a short distance from the ship. The time showed that solution of the salt had been more rapid than was anticipated.

The second test, on September 26, took place in a choppy and increasing sea. The net was shot at 12.45 p.m., with a release which under experimental conditions in running water should have lasted 3 hours but which was expected, after the previous experience, to last not more than two and a half hours. The flag was sighted at 3.25 p.m., a short distance away and picked up a few minutes later.

The catch from the first test appeared at first glance to be dis-

appointingly small. There were, however, thirty-two different species (Euphausians 3, Copepods 9, Siphonophores 9, Chaetognaths 6, Medusae 2, Tunicates 2, Fishes 1) eighteen of which were represented by single specimens (56%). In the second test the bucket produced forty-five different species (Euphausians 4, Copepods 13, Decapods 2, Siphonophores 11, Chaetognaths 7, Medusae 3, Tunicates 1, Polychaets (Tomopteris) 1, fishes 3) twenty-six of which were represented by single specimens (58%). The preliminary analysis of the two catches was made by Dr. H. B. Moore, who remarked on the perfect condition of such delicate organisms as *Calocalanus pavo* and noted that two records in the first catch and nine in the second were new to him from Bermuda.

We have no data at present from which to judge the comparative efficiency of the parachute net. It may be far below that of a standard townet of the same size. This, however, is beside the point. The net was not intended to supersede the towed net, or even to compete with it in its own field. Its special purpose is the capture of organisms for the study of life processes in the deep sea. The principle of the net having been proved, and the retention of organisms by the trap-bucket having been demonstrated, technical improvements may lead to the provision of living material such as could never have been obtained by the older methods. Two obvious alterations can be made in the present net. It is doubtful whether the secondary cone in the mouth serves a useful purpose since it is not part of the parachuting surface and the trapping of animals not in the bucket when the weight reaches the bottom is probably quite efficiently done by the collapse of the net and the folding up of the mouth. A single streamlined weight should increase the speed of the net. Further advance in design, however, lies in the construction of a much larger net and in improvements to the bucket. I suspect that the parachuting surface of the present net is really too great for the size of the mouth and that it would open a net of twice the diameter. The aerial parachute has a large parachuting surface because it is employed to land its load gently on the earth. In the net it has merely to keep open the mouth against the inward pull of the bridles and may thus be greatly diminished especially as it works in such a dense medium as water. Size and length of net and bridles can be increased to any extent within reason without increasing the practical difficulties of handling. Weight is the only restriction. A light impervious material instead of the painted calico could be used with advantage. The bucket in its present form should be jacketed so that the inner chamber remains cool and the catch should be refrigerated as soon as possible after reaching the surface.

With these improvements the net may bring the physiological processes of deep-sea organisms under direct observation and experiment; it will certainly provide specimens more perfect than previously known; it can be shot in deep water out of sight of land near a buoyed sea-anchor and fish the depths while routine work is carried on from the ship; and there is always a chance that rare or even new forms living close to the bottom may be captured.

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